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(71) Applicant *(for all designated States except US)*: **UNIVERSITY OF KENTUCKY RESEARCH FOUNDATION** [US/US]; A144, ASTeCC Building, University of Kentucky, Lexington, KY 40506-0286 (US).

(71) Applicant *(for US only)*: **LATUS, Lori, J.** (executor for the deceased inventor) [US/US]; 1227 Scoville Road, Lexington, KY 40517 (US).

(72) Inventor: **BURKE, Thomas, G.** (deceased).

(72) Inventor; and

(75) Inventor/Applicant *(for US only)*: **BOM, David, C.** [US/US]; 1000 Vineyard Drive, Apartment 203, Broadview Hts., OH 44147 (US).

(74) Agents: **SCHICKLI, Warren, D.** et al.; King & Schickli, PLLC, 247 North Broadway, Lexington, KY 40507 (US).

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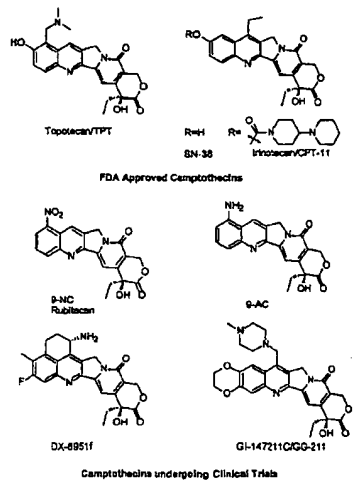
(54) Title: ENGINEERED LIPOSOMAL PARTICLES CONTAINING CORE-LOADED PRO-DRUGS FOR THE CONTROLLED RELEASE OF CAMPTOTHECINS



(57) Abstract: A liposome composition includes an entrapped camptothecin ester derivative loaded predominantly in the aqueous core of the liposomal particle. More particularly the invention relates to controlled release formulations that entail the liposomal core-loading of camptothecin-20-esters (CPT-20-OR). Through judicious choice of liposomal encapsulation methodologies, lipid ingredients, and the choice of the $R=CO[CH_2]_nNH_2HCl$ functionally of the CPT-20-OR, formulations are developed which release camptothecin pro-drug from the liposomal particle. Upon reaching the outside of the particle and gaining exposure to the physiological mileau (i.e. pH 6.8 to 7.4) at the tumor site, the glycinate esters decompose to generate the active lactone of camptothecins which are potent inhibitors of DNA topoisomerase I and potent anti-cancer agents.

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ENGINEERED LIPOSOMAL PARTICLES CONTAINING CORE-LOADED PRO-DRUGS FOR THE CONTROLLED RELEASE OF CAMPTOTHECINS

This application claims the benefit of U.S. Provisional Patent Application Serial No. 60/331,908 filed November 20, 2001.

Technical Field

The present invention relates to camptothecin pro-drugs comprising camptothecin-20-aminoester derivatives loaded into the aqueous core of a liposome. The invention further relates to methods for reducing toxicity and extending in vivo survival of a camptothecin or camptothecin analogue, comprising synthesizing camptothecin-20-aminoester derivatives and loading them into the aqueous core of a liposome. Still further, the invention relates to pharmaceutical compositions comprising the camptothecin pro-drugs as described, and to methods of inhibiting topoisomerase I and treating cancer in a mammal using the camptothecin pro-drugs.

Background of the Invention

Camptothecin and related analogs (Figure 1) represent an important class of agents useful in the treatment of cancer. The widespread clinical interest in the camptothecins stems from their unique mechanism of action: they stabilize the covalent binding of the enzyme topoisomerase I (topo I), an intranuclear enzyme that is overexpressed in a variety of tumor lines, to DNA.

This drug/enzyme/DNA complex leads to reversible, single strand nicks that, according to the fork collision model, are converted to irreversible and lethal double strand DNA breaks during replication. Therefore, due to the mechanism of its cytotoxicity, CPT is S-phase specific, indicating that it is only toxic to cells that are undergoing DNA synthesis. Rapidly dividing cells, such as cancerous cells, spend more time in the S-phase relative to healthy tissues. Thus, the overexpression of topo I, combined with the faster rate of mitosis, provide a limited basis for selectivity via which camptothecins can effect cytotoxicity on cancerous cells rather than healthy host tissues.

As a class, the camptothecins have exhibited unique dynamics and reactivity in vivo, both with respect to drug hydrolysis and blood protein interactions. These factors have confounded their pharmaceutical development and clinical implementation. In terms of hydrolysis, each of the clinically relevant camptothecins shown in Figure 1 contains an α-hydroxy-δ-lactone pharmacophore; at pH 7 and above this functionality is highly reactive and readily converts to the "ring opened" carboxylate form (as is shown for camptothecin in Figure 2). Unfortunately, the carboxylate form of the camptothecin agent is inactive. Thus, as a result of the labile α-hydroxy-δ-lactone pharmacophore, camptothecins exist in an equilibrium consisting of two distinct drug species: 1) the biologically active lactone form where the

lactone ring remains closed; and 2) a biologically-inactive carboxylate form generated upon the hydrolysis of the lactone ring of the parent drug.

This hydrolysis problem with camptothecin and many analogs (*e.g.* 9-aminocamptothecin, 9-nitrocamptothecin) is exacerbated in human blood. In human blood and tissues, the camptothecin equilibrium of active lactone form vs. inactive carboxylate form can be greatly affected by the presence of human serum albumin (HSA). Time-resolved fluorescence spectroscopic measurements taken on the intensely fluorescent camptothecin lactone and camptothecin carboxylate species have yielded direct information on the differential nature of these interactions with HSA. The lactone form of camptothecin binds to HSA with moderate affinity yet the carboxylate form of camptothecin binds tightly to HSA, displaying a 150-fold enhancement in its affinity for this highly abundant serum protein. Thus, when the lactone form of camptothecin is added to a solution containing HSA, the preferential binding of the carboxylate form to HSA drives the chemical equilibrium to the right, resulting in the lactone ring hydrolyzing more rapidly and completely than when camptothecin is in an aqueous solution without HSA.

These dynamic processes present a major hurdle to achieving successful chemotherapy for a cancerous disease state. In addition to modulating human blood stability, it has been shown that the presence of physiologically relevant concentrations of HSA can greatly attenuate (by

several orders of magnitude) the anticancer activities (IC_{50} values) of these agents. In humans it appears that protein binding interactions make it difficult to achieve therapeutically effective unbound lactone levels of these agents, particularly when one considers that continuous exposures (for tumor cells to cycle through S-phase) of the active lactone form are requisite for efficacy purposes.

Liposomes have been shown to provide an excellent means by which to stabilize the biologically-active lactone form of camptothecins. Water-soluble drugs such as topotecan can be entrapped within the pH-adjusted aqueous compartments of liposomes with the acidic microclimate of liposomes stabilizing the active lactone form. Lipophilic drugs can partition into the bilayer where the lactone ring is stabilized and protected from hydrolysis. Liposomes can also serve as controlled release depots. The camptothecins are S-phase specific drugs, and it has been shown that optimal activity is obtained when the tumors of a patient are exposed to the drugs for continuous periods of time. Liposomes that target tumors and slowly release drug (such that tumor cells are continuously exposed to drug) appear as attractive drug delivery systems to pursue.

The first FDA approval of a liposomal anticancer product was liposome-encapsulated doxorubicin (Doxil) from SEQUUS (now ALZA). In early 1996 liposome-encapsulated daunorubicin (DaunoXome) from Nexstar

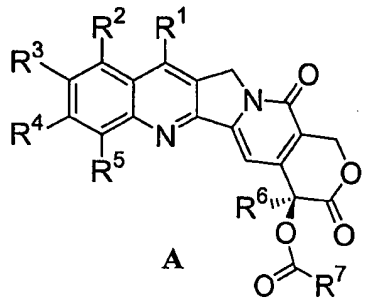
(now Gilead) was also approved for marketing. Both products use unilamellar liposome configurations composed of phosphatidylcholines with saturated fatty acid chains and cholesterol as their basic ingredients. These lipids in combination create a durable bilayer that prevents the leakage of drug from the vesicle. The tough bilayer also deters the opsonization process from occurring. The Doxil formulation includes 5% polyethyleneglycol (PEG)-linked distearoyl-phosphatidyethanolamine in the liposome, which effectively extends the lifetime of the liposome in circulation. Studies have shown that the Doxil and Daunoxome products pass through the fenestrated vasculature of growing tumors; thus, the leaky nature of the vasculature promotes drug accumulation. An excellent example of this is Kaposi's sarcoma, a common cancer in patients with AIDS, that is known to respond well to tumor-targeted liposomes; the enhanced accumulation of drug at the tumor site has been directly attributed to the leaky blood vessels that are characteristic of Kaposi's sarcoma. The successful use of the Doxil and Daunoxome medications described above (and other favorable clinical results obtained with TLC-99 liposomal doxorubicin) has been achieved as a result of 30 years of intensive and persistent research and development.

Clearly, the past several years have seen rapid advancement and progress in both the camptothecin drug development field as well as the liposomal drug delivery arena. A number of companies have been active in

the liposomal formulations of camptothecins in recent years. The lead liposomal camptothecin is GG 211. Both Gilead and ALZA developed liposomal formulations of this agent. Both companies reported encouraging findings that liposomal formulation resulted in 3 to 5-fold gains in the efficacy of the agents against human cancers carried in murine models. In addition to GG211, Inex has reported favorable results in formulating topotecan in liposomes, showing a significant improvement in efficacy through the liposomal formulation process. There is also significant interest in liposomal aerosol formulations of 9-AC and 9-NC, although these drugs only load into the bilayer compartment of liposomes since they lack a basic amino functionality necessary for core-loading. Core-loaded liposomal formulations of CPT-11 have also been prepared and evaluated. Liposomal core-loading of camptothecins has been limited to date to agents such as topotecan, CPT-11, GG-211, and CKD602 which actively load using ion gradients into pre-made liposomes.

Summary of the Invention

In accordance with the purposes of the present invention as described herein, the present invention provides a composition comprising a camptothecin-20-aminoester derivative having the structure:



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wherein R^1 may be hydrogen, a halogen atom, a branched or linear alkyl group, a branched or linear alkenyl group, a C_{3-7} cycloalkyl group, a branched or linear alkynyl group, an alkoxy group, an alkylamino group, a dialkylamino group, an alkylthiol group, a thiol group, a phenyl group, an amino group, a nitro group, a cyano group, or $(CH_2)_YNR_8R_9$, wherein: (a) Y is an integer from 1-10 and R_8 and R_9 are, independently, hydrogen, an alkyl group, an alkenyl group , an alkynyl group, an amine, an alkyl amine, a dialkyl amine, a hydroxy group, an alkoxy group, an acyl group, or a carbamate, and (b) R_8, R_9 and the nitrogen to which they are attached may form a saturated or unsaturated three- to ten-membered heterocyclic ring containing O, S, and NR^{10} wherein R^{10} is a hydrogen, an alkyl group, an alkenyl group, an alkynyl group, an alkoxy group or a carbamate. R^1 may also be a C_{1-10} cycloalkyl group, a C_{1-10} cycloalkenyl group, or a C_{1-10} cycloalkynyl group.

R^2 may be hydrogen, a halogen atom, a linear or branched alkyl group, a linear or branched alkenyl group, a linear or branched alkynyl group, an amino group, an alkylamino group, a dialkylamino group, a nitro group, a 3-10 membered heterocyclic ring, a C_{3-10} cycloalkyl group, a C_{3-10} cycloalkenyl group, a C_{3-10} cycloalkynyl group, a thiol group, or a cyano group. R^2 may also be $(CH_2)_YNR_8R_9$, wherein: (a) Y is an integer from 1-10; and (b) R_8 and R_9 are, independently, hydrogen, an alkyl group, an alkenyl group , an alkynyl group, an amine, an alkyl amine, a dialkyl amine, a hydroxy group, an alkoxy group, an acyl group, or a carbamate; and (c) R_8, R_9 and the nitrogen to which they are attached may form a saturated or unsaturated three to ten membered heterocyclic ring.

R^3 may be hydrogen, a halogen atom, a linear or branched alkyl group, a linear or branched alkenyl group, a linear or branched alkynyl group, an amino group, an alkylamino group, a dialkylamino group, a nitro group, a 3-10 membered heterocyclic ring, a C_{3-10} cycloalkyl group, a C_{3-10} cycloalkenyl group, a C_{3-10} cycloalkynyl group, a thiol group, a cyano group, a hydroxyl group, or may be a compound having the formula $(CH_2)_YNR_8R_9$, wherein: (a) Y is an integer from 1-10; (b) R_8 and R_9 are, independently, hydrogen, an alkyl group, an alkenyl group, an alkynyl group, an amine, an alkyl amine, a dialkyl amine, a hydroxy group, an alkoxy group, an acyl group, a carbamate; and (c) R_8, R_9 and the nitrogen to which they are attached may form a saturated or

unsaturated three to ten membered heterocyclic ring.

R^4 may be a hydrogen, a halogen atom, a hydroxy group, an amino group, a methoxy group, an alkyl group, an alkynyl group or an alkenyl group. R^5 may be hydrogen or fluorine. R^6 may be an alkyl group, an alkenyl group, an alkynyl group, or a benzyl group. R^7 may be a side chain of a naturally occurring amino acid, or a compound having the formula $(CH_2)_LNR^{14}R^{15}$, wherein L is an integer ranging from 1-30 and R^{14} and R^{15} are independently the same or different and are hydrogen, a C_{1-15} alkyl group, a C_{2-15} alkenyl group, a C_{2-15} alkynyl group or an aryl group.

R^1 may be linked to R^2 in accordance with the structure $R^1(CH_2)_QR^2$, wherein Q represents an integer 1-10, a $SiR_{11}R_{12}R_{13}$ group. Q may also represent a compound having the formula $(CH_2)_FSiR_{11}R_{12}R_{13}$, wherein F is an integer from 1-10, and R_{11}, R_{12} and R_{13} independently represent hydrogen, a halogen atom, an alkyl group, an alkenyl group, an alkynyl group, a C_{3-10} cycloalkyl group, a C_{3-10} cycloalkenyl group, a C_{3-10} cycloalkynyl group, an amino group, or a hydroxy group. R^2 may be linked to R^3 in accordance with the structure $R^2(CH_2)_GR^3$, wherein G is an integer from 1-10, and one or more N, O or S atoms are substituted for one or more $-CH_2-$ groups.

R^3 may be linked to R^4 in accordance with the structure $R^3(CH_2)_GR^4$ wherein G is an integer from 1-10, and one or more N, O or S atoms are substituted for one or more $-CH_2-$ groups. Typically, L will be an integer

ranging from 1-6.

Suitable camptothecins from which the camptothecin-20-aminoester derivative may be derived include camptothecins selected from the group consisting of SN-38, 9-aminocamptothecin, DX-8951f, GG-211, 9-nitrocamptothecin, topotecan, CPT-11, lurtotecan, CKD-602, 10-hydroxycamptothecin, and ST1481.

In another aspect, the present invention provides a method for reducing toxicity of a camptothecin, comprising the steps of synthesizing a camptothecin-20-aminoester pro-drug, and incorporating the camptothecin-20-aminoester pro-drug into an aqueous core of a liposome. The camptothecin-20-aminoester pro-drug may be selected from compounds having the structure as described above, and may be synthesized from any camptothecin as described above.

In yet another aspect, the present invention provides a method for extending in vivo survival of a camptothecin comprising the steps of synthesizing a camptothecin-20-aminoester pro-drug having the structure as described above, and incorporating the camptothecin-20-aminoester pro-drug into an aqueous core of a liposome. The camptothecin-20-aminoester pro-drug may be synthesized from the group of camptothecins as described above.

In still yet another aspect of the present invention, a pharmaceutical composition is provided, comprising an amine-containing camptothecin

derivative incorporated into an aqueous core of a liposome. The amine-containing camptothecin derivative is typically a camptothecin-20-aminoester having a structure as described above. The pharmaceutical composition may be derived from any of the group of camptothecins as described above.

In still yet another aspect of the present invention, a method of forming a topoisomerase I-inhibiting camptothecin compound in a mammal is provided, comprising administering a liposome preparation comprising a liposome containing a camptothecin-20-aminoester derivative in an aqueous core. The liposome preparation is administered to a mammal in an amount sufficient to inhibit topoisomerase I. The camptothecin-20-ester may have the structure as substantially described above, and may be derived from any of the group of camptothecins as described above. Typically, a sufficient amount of the liposome preparation of the present invention will be administered to provide from about 1 to about 200 mg/kg body weight per week of the camptothecin-20-aminoester derivative. The liposome preparation may be administered parentally.

In still yet another aspect of the invention, a method of treating cancer in a mammal, including a human, is provided comprising administering an effective amount of a liposome preparation. The liposome preparation may comprise a liposome containing a camptothecin-20-aminoester derivative having the structure as described above in the aqueous core of the liposome.

The camptothecin-20-aminoester derivative may be derived from a camptothecin selected from the group consisting of SN-38, 9-aminocamptothecin, DX-8951f, GG-211, 9-nitrocamptothecin, topotecan, CPT-11, lurtotecan, CKD-602, 10-hydroxycamptothecin, and ST1481 as described above. Typically, a sufficient amount of the liposome preparation of the present invention will be administered to provide from about 1 to about 200 mg/kg body weight per week of the camptothecin-20-aminoester derivative. The liposome preparation may be administered parentally.

Other objects and applications of the present invention will become apparent to those skilled in this art from the following description wherein there is shown and described a preferred embodiment of this invention, simply by way of illustration of the modes currently best suited to carry out the invention. As it will be realized, the invention is capable of other different embodiments and its several details are capable of modification in various, obvious aspects all without departing from the invention. Accordingly, the drawings and descriptions will be regarded as illustrative in nature and not as restrictive.

Brief Description of the Drawings

The accompanying drawing incorporated in and forming a part of the specification illustrates several aspects of the present invention and, together with the description, serves to explain the principles of the invention. In the

drawing:

Figure 1 shows the structures of clinical candidates and FDA-approved analogs in the camptothecin family of antitumor agents.

Figure 2 schematically depicts camptothecin hydrolysis at physiological pH.

Figure 3 shows a structural comparison between SN-38 and DB-67.

Figure 4 schematically depicts the two compartments for drug loading that are contained within a lipid vesicle. The top panel represents the situation where an entrapped drug loads predominantly into the lipid bilayer compartment; the lower panel represents drug loading into the aqueous cavity contained in the core of the liposomal particle.

Figure 5 shows the mechanism of active drug loading in a liposomal particle, whereby an amine-containing agent loads into the particle by a gradient caused by ammonia gas diffusing out of the particle, thereby creating a driving force for an amine-containing compound to enter the liposome. Upon being suspended in blood, forces act on the drug and the liposome promoting release of the agent.

Figure 6 schematically depicts the structures of camptothecin and related camptothecin-20-ester analogs displaying enhanced E-ring lactone stability.

Figure 7 shows a HPLC chromatogram depicting the separation of camptothecin from camptothecin carboxylate.

Figure 8 shows a HPLC chromatogram depicting the markedly improved solution stability of camptothecin-20-acetate relative to camptothecin as shown in Figure 7.

Figure 9 illustrates the improved human blood stability of camptothecin-20-acetate relative to camptothecin in phosphate buffered saline and in human blood (1 μM drug concentration).

Figure 10 shows HPLC chromatograms depicting the high purity and high stability in non-aqueous DMSO solution of a hydrochloride salt preparation (left panel) and a trifluoroacetate salt preparation (right panel) of camptothecin-20-glycinate ester.

Figure 11 shows HPLC chromatograms depicting the pronounced and instantaneous reactivity of camptothecin-20-glycinate ester hydrochloride upon addition to PBS buffer under near physiological conditions of ionic strength and temperature.

Figure 12 shows HPLC chromatograms depicting the pronounced and instantaneous reactivity of camptothecin-20-glycinate ester hydrochloride upon addition to human blood.

Figure 13 illustrates the pronounced reactivity of camptothecin-20-glycinate ester in human plasma (left panel) and human blood (right panel) at an initial pro- drug concentration of 1 μM.

Figure 14 shows the impact of pH on the chemical decomposition of

camptothecin-20-glycinate ester in PBS buffer at 37 °C at a pro-drug concentration of 1 μM.

Figure 15 shows the effect of pH on the reversibility of the formation of the chemical degradation products of camptothecin-20-glycinate ester in PBS buffer at 37 °C at a pro-drug concentration of 1 μM.

Figure 16 schematically depicts reaction mechanisms which camptothecin-20-glycinate ester hydrochloride undergoes in aqueous solution at 37 °C at a pro-drug concentration of 1 μM.

Figure 17 shows stability profiles of several different camptothecin pro-drug structures in PBS buffer.

Figure 18 shows stability profiles of several different camptothecin pro-drug structures in whole blood. Data sets are shown for both free drugs in whole blood as well as core-loaded pro-drugs.

Figure 19 shows normalized fluorescence emission spectra of 1 μM of camptothecin-20-glycinate ester hydrochloride in PBS buffer at different pH values.

Figure 20 shows fluorescence excitation and emission spectra of 1 μM of camptothecin-20-glycinate ester hydrochloride in PBS buffer in the presence and absence of 0.1 M dimyristoylphosphatidylcholine (DMPC) or 0.1 M dimyristoylphosphatidlyglycerol (DMPG) small unilamellar liposomes.

Figure 21 illustrates the associations of camptothecin-20-glycinate ester to small unilamellar vesicles composed of electroneutral DMPC (Panel A) and DMPG (Panel B) suspended in phosphate buffered saline, using fluorescence anisotropy titration.

Figure 22 shows the pronounced reactivity of camptothecin-20-glycinate ester in the presence of varying concentrations of DMPC SUVs in PBS buffer at 37 °C at pH 7.4 at an initial pro-drug concentration of 1 μM.

Figure 23 shows the pronounced reactivity of camptothecin-20-glycinate ester in the presence of varying concentrations of DMPC SUVs in PBS buffer at 37 °C at pH 10 (top panel) and pH 3.0 at an initial pro-drug concentration of 1 μM.

Figure 24 illustrates the pronounced stabilization in PBS (left panel) and whole human blood (right panel) which core loading of camptothecin-20-glycinate ester hydrochloride into DSPC liposomes provides for pro-drug stability at 37 °C at pH 7.4.

Figure 25 shows the pronounced stabilization in PBS (left panel) and whole human blood (right panel) provided by core loading of camptothecin-20-glycinate ester trifluoroacetate into DSPC liposomes has on pro-drug stability at 37 °C at pH 7.4.

Figure 26 provides a comparison of the model membrane associations of several A-ring and A,B-ring modified camptothecin

glycinate ester compounds to small unilamellar vesicles (SUVs) composed of electroneutral dimyristoylphosphatidylcholine (DMPC) and negatively-charged dimyristoylphosphatidylglycerol (DMPG) in PBS. Data sets are also shown for camptothecin-20-glycinate ester, 7-chloromethyl-10,11-methylenedioxy-camptothecin-20-glycinate ester, 9-chloro-10,11-methylenedioxycampto-thecin-20-glycinate ester, and 7-ethyl-10,11-methylenedioxycamptothecin-20-glycinate ester binding to DMPC and DMPG bilayers.

Figure 27 provides a comparison of the equilibrium binding of several E-ring modified camptothecin-20-OR esters (where R=$CO[CH_2]_n NH_2HCl$ where n= 1-3) to small unilamellar vesicles (SUVs) composed of electroneutral dimyristoylphosphatidylcholine (DMPC) and negatively-charged dimyristoylphosphatidylglycerol (DMPG) in PBS. Data sets are also shown for camptothecin-20-glycinate ester, camptothecin-20-propanate ester, and camptothecin-20-butanate ester. Data for camptothecin is included for comparative purposes.

Figure 28 shows the reactivity of highly lipophilic 7-t-Butyldimethylsilyl-10-hydroxycamptothecin glycinate ester (DB-67 glycinate) in PBS buffer (left panel) and human blood (right panel) at an initial pro-drug concentration of 1 μM.

Figure 29 shows a comparison of the reactivities of several E-ring modified camptothecin-20-OR esters (where R=$CO[CH_2]_nNH_2HCl$ where n= 1-3) in PBS buffer (left panel) and human blood (right panel) at an initial pro-drug concentration of 1 μM.

Figure 30 depicts the reactivity of camptothecin-20-propanate ester hydrochloride in the presence of varying concentrations of DMPC SUVs in PBS buffer at 37 °C at pH 7.4 at an initial pro-drug concentration of 1 μM.

Figure 31 illustrates the reactivity of camptothecin-20-butanate ester hydrochloride in the presence of varying concentrations of DMPC SUVs in PBS buffer at 37 °C at pH 7.4 at an initial pro-drug concentration of 1 μM.

Figure 32 shows the markedly improved stability of core-loaded liposomal camptothecin-20-propanate in PBS buffer relative to the corresponding data set for camptothecin-20-glycinate.

Figure 33 summarizes the markedly improved stability of liposomal core-loaded camptothecin-20-propanate ester in PBS versus bilayer-loaded camptothecin-20-propanate ester.

Figure 34 shows the markedly improved stability of core-loaded liposomal camptothecin-20-butanate pro-drug dispersed in human blood versus drug in its free unencapsulated form.

Figure 35 illustrates the stability of core-loaded liposomal DB-67 glycinate dispersed in human blood.

Figure 36 illustrates the stability of DB-67 butanate (free drug) in PBS buffer and whole human blood.

Figure 37 summarizes the markedly improved stability of core-loaded liposomal camptothecin-20-glycinate ester in PBS.

Figure 38 shows the markedly improved solution stability of liposomal DB-67 butanate ester in whole human blood.

Reference will now be made in detail to the presently preferred embodiments of the invention, examples of which are illustrated in the accompanying drawing.

Detailed Description of the Invention

In the present patent application we demonstrate how opportunities in the areas of camptothecin rational design and liposomal formulation can be used to engineer controlled release vehicles for camptothecins. The methods and compositions of the present invention may be accomplished by various means which are illustrated in the examples below. These examples are intended to be illustrative only, as numerous modifications and variations will be apparent to those skilled in the art.

We have developed particles that contain camptothecin pro-drugs that readily core-load, even if the parent active agent is highly lipophilic.

This invention provides a means of preparing tumor-targeted liposomes containing pro-drugs of camptothecin and related analogs.

The simple, versatile and tractable chemically activated pro-drug (CAP) approach of the present invention can be applied to essentially any camptothecin including the agents summarized in Figure 1. Many companies have tried to prepare core-loaded liposomal formulations of specific camptothecins but have been unable to do so because the active agent did not possess an amino group of sufficient basic character to allow for core-loading by active loading methods. Significant but unsuccessful efforts were made by a number of labs to attempt to core-load 9-AC (an agent that was evaluated extensively in humans but apparently failed in the clinic largely because of its highly specific interactions with human albumin and its resultant poor human blood stability). ALZA and others have been unable to succeed with generating core-loaded liposomal 9-AC formulations. Conversion of 9-AC to a CAP (via esterification of a glycine) should readily allow the agent to core-load. In light of the extensive human data on the 9-AC agent (trials were terminated at the end of phase III), it seems logical to examine the potential of liposomal 9-AC CAP. Another way the CAP approach could find commercial interest is to further optimize drug retention in the core of liposomes. Gilead has developed GG211 core-loaded in liposomes; results by this lab and several others have shown that

GG211 leaks from the core of NX-211 liposomes. Gilead now has their core-loaded liposomal GG211 product in phase II clinical trials, but they are reports of hematological toxicities; these toxicities may be due to premature leakage of the agent into the blood prior to targeting at the tumor site. Our CAP approach may have two advantages for the Gilead product: 1) it would create an inactive prodrug that, in the case of leakage in the blood, would potentially reduce toxicity; and 2) the additional positive charge carried by the CAP side-chain may significantly aid in retaining the drug in the particle until release occurs at the tumor site. CKD602 is now being formulated in the core of liposomes by ALZA/Johnson and Johnson, and conversion of CKD602 into a pro-drug may provide for particle retention advantages.

CAP is a versatile approach in that it can be used on any of the existing camptothecins such as SN-38, 9-AC, 9-NC, and GG-211. The CAP pro-drug ester approach focuses on position 20(S) of the E-ring and entails the esterification with the amino acid glycine or larger $R=CO[CH_2]_n NH_2HCl$ functionalities. The synthesis of this type of camptothecin pro-drug ester has been previously described by Wall and Wani (PCT Patent Application S.N. WO09602546A1) and Vishnuvajjala and Garzon-Aburbeh (U.S. Patent No. 4,943,579). We have shown that the CAP 20(S)-glycinate ester is spontaneously converted to the parent agent through a lactam intermediate. We have shown that the longer chain esters propanate and

butanate react at a much slower rate than glycinate (because the resulting seven- and eight- membered lactams are significantly less stable than the six-membered lactams formed from glycinate ester decomposition). We have also demonstrated the pro-drugs are significantly more stable at low pH, indicating that a low pH environment within the liposomal particle would be favorable in maintaining the pro-drug intact.

Our specific CAP technology relates to a liposome composition having entrapped parent camptothecin ester derivatives loaded predominantly in the aqueous core of the particle. Through judicious choice of liposomal encapsulation methodologies, lipid ingredients, and the choice of the $R=CO[CH_2]_n NH_2HCl$ functionality of the camptothecin-20-ester, formulations have been described which release camptothecin pro-drug from the liposomal particle to generate active parent camptothecin in a rational and controlled fashion resulting in optimal therapeutic efficacy. Upon departing the liposomal particle at the tumor site the camptothecin - 20-ester undergoes spontaneous decomposition and generates the active lactone forms of camptothecins which are potent inhibitors of DNA topoisomerase I and potent anti-cancer agents. Tumor-targeting will allow for a reduction of systemic toxicity of the encapsulated agent. Thus, the availability of the liposomal CAP described in this provisional patent application provides convenient handles on controlling the rate of formation

of active camptothecin; using these various delivery systems the optimal rate of camptothecin parent drug release at the tumor site for optimal regression to occur can be achieved.

The parent structure camptothecin is water-insoluble and hence a number of investigators have pursued the development of more water-soluble agents. U.S. Patent No. 4,943,579 by Vishnuvajjala and Garzon-Aburbeh discloses water-soluble derivatives of camptothecins having the formula CPT(20)-OR where R=COCH(2)NH(2)HCl. U.S. Pat. No. 4,943,579 discloses the esterification of the hydroxyl group at the 20-position of camptothecin to form several pro-drugs. This patent further discloses that the pro-drugs are water soluble and are converted into the parent camptothecin compounds by hydrolysis.

Following the above disclosure by Vishnuvajjala and Garzon-Aburbeh, Wall and Wani in U.S. Pat. Nos. 5,916,896, 5,646,159, and 6,040,313 disclose that esterification of the hydroxyl group at the 20-position of camptothecin compounds produces a non-toxic water-soluble pro-drug. Wall and Wani claim the pro-drug is non-toxic even though the parent camptothecin compound itself may be substantially more toxic. Wall and Wani teach hydrolysis of the ester formed at the 20-position reforms the parent camptothecin compound after administration thereby reducing the overall toxicity experienced by the patient during camptothecin therapy.

Wall and Wani studied the toxicity or non-toxicity of the camptothecin esters by monitoring weight loss in test animals such as mice which have been administered the ester compounds. By "non-toxic", Wall and Wani teach the glycinate ester compounds of camptothecin are not toxic according to Protocol 4, section 4.301(b)(3) where toxicity is defined as a weight loss of 4.0 grams as reported in R. I. Geran, N. H. Greenberg, M. M. MacDonald, A. M. Schumacher and B. J. Abbott, Cancer Chemotherapy Reports, Part 3, Vol. 3, No. 2, September 1972 (incorporated herein by reference). Wall and Wani teach that pro-drugs formed by esterifying the hydroxyl group at the 20-position are non-toxic in contrast to the toxicity of parent camptothecin compounds even though the esterified derivatives are hydrolyzed to the parent camptothecin compounds after administration. Wall and Wani in U.S. Pat. No. 4,943,579 do not suggest that pro-drugs formed by esterifying the hydroxyl group at the 20-position are non-toxic relative to the parent compounds, and claim the compounds disclosed in U.S. Pat. No. 4,943,579 are not within their inventions described in U.S. Pat. Nos. 5,916,896, 5,646,159, and 6,040,313.

It is important to note that both Vishnuvajjala and Garzon-Aburbeh and Wall and Wani teach that hydrolysis of the ester formed at the 20-position reforms the parent camptothecin compound after administration. Wall and Wani claim that hydrolysis of the exocyclic ester bond in vivo

regenerates the parent hydroxyl group containing camptothecin compound. No additional mechanistic detail is given as to how this hydrolysis occurs. Alkyl esters have also been described by Cao and Giovanella also contain an exocyclic ester bond and no information is given by Wall and Wani as to how the hydrolysis of these two classes of camptothecin esters vary in terms of the hydrolysis of the exocyclic ester (OC=O) bond. Wadkins et al. also teach that camptothecin glycinate esters also hydrolyze, but no information is given in their work as to how the exocyclic ester bonds of camptothecin glycinate esters and camptothecin alkyl esters vary. Further, Wall and Wani claim in their patent R=$CO[CH_2]_n NH_2HCl$ where n= from a short value of 1 to a long value such as 12 with no discussion whatsoever of how extension of this chain would impact on hydrolysis. Wall and Wani teach that the compounds of their invention can be administered in the form of liposome or microvesicle preparations. They describe liposomes as microvesicles which encapsulate a liquid within lipid or polymeric membranes. Liposomes and methods of preparing liposomes are known and are described by Wall and Wani, for example, in U.S. Pat. No. 4,452,747, U.S. Pat. No. 4,448,765, U.S. Pat. No. 4,837,028, U.S. Pat. No. 4,721,612, U.S. Pat. No. 4,594,241, U.S. Pat. No. 4,302,459 and U.S. Pat.No. 4,186,183. The disclosures of these U.S. patents are incorporated herein by reference. Suitable liposome preparations for use in the present invention are also

described in WO-9318749-W, J-02056431-A and EP-276783-A, also incorporated herein by reference, No mention is made by Wall and Wani as to how the esters with the $R=CO[CH_2]_n NH_2HCl$ functionality where n= 1 to a long value such as 12 differ in their interactions with liposome formulations. Further, no discussion is made as to how the esters with the $R=CO[CH_2]_n NH_2HCl$ functionality interact with the lipid bilayers of the liposomal formulations.

In 1992 it was discovered that the active lactone forms of camptothecins could be readily stabilized by using liposomal carriers (Burke, U.S. Patents 5,552,156 and 5,736,156, incorporated herein by reference). Liposomes contain both aqueous and lipid bilayer compartments, and it was shown that both of these compartments could be utilized to stabilize the lactone form of the drug. All of the camptothecins studied to date display some degree of lipophilicity, and some fraction of the drug encapsulated within a liposomal formulation will be located within the lipid bilayer at any given time. The bilayer-localized fraction of the camptothecins preferentially partition as the active lactone form. In this manner the lipid bilayer compartment of a liposome contributes to stabilizing the active lactone form of a camptothecin. In 1992 it was also disclosed that agents displaying reduced lipophilicity could be further stabilized by reducing the pH of the internal aqueous core (Burke, U.S. Patents 5,552,156 and 5,736,156). The

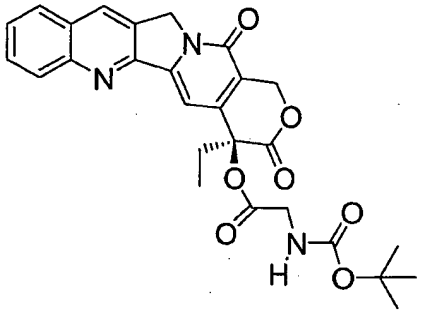
reduced pH of the internal core prevents hydrolysis of drugs localizing in the internal aqueous compartments and not within the bilayer compartment.

Liposome delivery systems can thus aid in solving an inherent shortcoming of this important class of anticancer agents by enhancing drug stability and anticancer activity. The drug-laden liposomal particles can be administered to patients and prolonged plasma exposure has been achieved. The particles can be passively or actively targeted to tumor using methods known in the art and, as a consequence, elevated active lactone levels of the drug can be realized at this site.

Liposomes can also serve as controlled release depots. As previously stated, the camptothecins are S-phase specific drugs, and it has been shown that optimal activity is obtained when the tumors of a patient are exposed to the drugs for continuous periods of time. This approach allows the drug to be present as the cancer cells cycle through S-phase. Liposomes that target tumors and slowly release internally contained drugs (such that tumor cells are continuously exposed to the desired drug) appear to be attractive drug delivery systems to pursue since they likely provide slow, continuous drug release to the tumor. The combination of internalizing a non-toxic camptothecin pro-drug that spontaneously activates at the tumor (at rates controllable by changing the alkyl linkage length between the ester bond and the amine) and

the release determined by the bilayer composition result in novel controlled release carriers that possess an added advantage of being tumor-targeted.

Example 1



Name: *tert*-Butoxycarbonylamino-acetic acid 4-ethyl-3,13-dioxo-3,4,12,13-tetrahydro-1*H*-2-oxa-6,12*a*-diaza-dibenzo[*b*,*h*]fluoren-4-yl-ester **(1)**

Molecular Weight: 505 g/mol

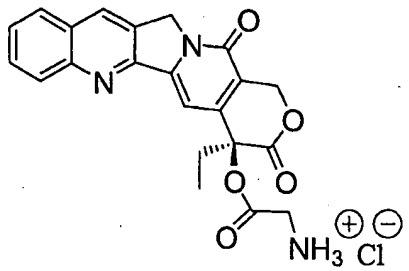
Molecular Formula: $C_{27}H_{27}N_3O_7$

Calculated Log P: 2.3352

Procedure:

Camptothecin(CPT) (1.00 g, 2.8 mmol) was placed in an oven dried flask under Nitrogen. Next anhydrous DMF (25 ml) was added followed by *N*-(*tert*-butoxy-carbonyl)glycine (1.06 g, 6.1 mmol), DMAP (0.189 g, 1.5 mmol) and DCC (1.20g, 5.82 mmol) generating a yellow slurry. After 5h at 22 °C the reaction became clear and the DMF was concentrated without heating under high vacuum. Next dichloromethane (25x3 ml) was added and the mixture was filtered each time. Finally, the crude material was purified by flash chromatography (99:1 CH_2Cl_2/acetone; 97:3 CH_2Cl_2/acetone; 95:5 CH_2Cl_2/acetone; 9:1 CH_2Cl_2/acetone) to provide **(1)** 482 mg (34% yield) as a light yellow solid: ^{1}H NMR (CD_2Cl_2) 300 MHz δ 1.01 (t, J= 7 Hz, 3 H), 1.406 (s, 9 H), 2.03-2.25 (m, 2 H), 4.06 (dd, J_1= 18 Hz, J_2= 6 Hz, 1 H), 4.20 (dd, J_1= 18 Hz, J_2= 6 Hz, 1 H), 5.11 (d, J= 19 Hz, 1 H) 5.19 (d, J= 19 Hz, 1 H), 5.30-5.43 (m, 2 H), 5.64 (d, J= 17 Hz, 1 H), 7.26 (s, 1 H), 7.62(t, J= 7 Hz, 1 H), 7.78 (t, J= 7 Hz, 1 H), 7.91 (d, J= 7 Hz, 1 H), 8.14 (d, J= 8 Hz, 1 H), 8.33 (s, 1H); ^{13}C NMR (CD_2Cl_2) 100 MHz δ 8.0, 28.6, 32.0, 43.0, 50.4, 67.4, 77.3, 80.3, 96.4, 120.0, 128.1, 128.5, 128.6, 129.1, 129.8, 130.8, 131.5, 146.0, 146.7, 149.0, 152.7, 156.1, 157.5, 167.6, 170.0.

Example 2



Name: 4-Ethyl-3,13-dioxo-3,4,12,13-tetrahydro-1*H*-2-oxa-6,12a-diaza-dibenzo[*b*,*h*]-fluoren-4-yloxycarbonylmethyl-ammonium chloride **(2)**

Molecular Weight: 442 g/mol

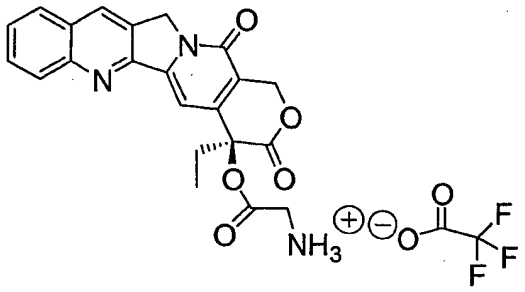
Molecular Formula: $C_{22}H_{20}ClN_3O_5$

Calculated Log P (on salt w/o counterion): 0.5394

The Boc protected glycinate ester of camptothecin **(1)** (85 mg, 0.30 mmol) was placed in an oven dried flask. Next a solution of hydrogen chloride in dioxane (33 ml, 4.0 M) was added dropwise generating a bright yellow mixture. After 3h the solvent was evaporated, the residue was washed with ether (3x5 ml) and pumped on overnight providing **(2)** as a bright yellow solid weighing 68 mg (92% yield): ^{1}H NMR (d_6-DMSO) 400 MHz δ 0.95 (t, *J*= 7 Hz, 3 H), 2.06-2.23 (m, 2 H), 4.01-4.06 (m, 1 H), 4.28-4.38 (m,1 H), 5.26-5.38 (m, 2 H), 5.55 (s, 2 H), 7.32 (s, 1 H), 7.74 (ddd, J_1= 8 Hz, J_2= 7 Hz,

J_3= 1 Hz, 1 H), 7.88 (ddd, J_1= 8 Hz, J_2= 7 Hz, J_3= 1 Hz, 1 H), 8.15-8.18 (m, 2 H), 8.46-8.62 (br m, 2 H), 8.73 (s, 1 H).

Example 3



Name: 4-Ethyl-3,13-dioxo-3,4,12,13-tetrahydro-1*H*-2-oxa-6,12a-diaza-dibenzo[*b*,*h*]-fluoren-4-yloxycarbonylmethyl-ammonium trifluoroacetate **(3)**

Molecular Weight: 535 g/mol

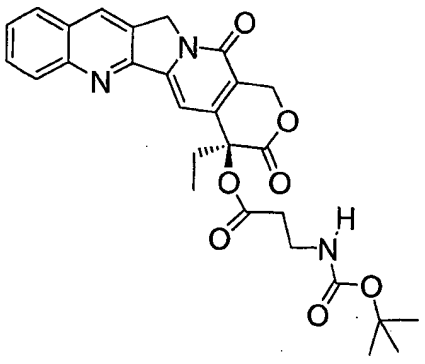
Molecular Formula: $C_{25}H_{24}F_3N_3O_7$

Calculated Log P: 0.5394

Camptothecin ester **(1)** (20 mg, 0.04 mmol) was placed in an oven dried flask under nitrogen and anhydrous CH_2Cl_2 (0.5 ml) was added. Next TFA (0.5 ml) was added dropwise at 0 °C. After 5 h at 22 °C the reaction

mixture was concentrated providing a light brown oily residue. The residue was washed with ether (3x1 ml) and placed under high vacuum overnight providing **(3)** as a bright yellow solid weighing 21 mg (98% yield): ^{1}H NMR (d_6-DMSO) 400 MHz δ 0.95 (t, *J*= 7 Hz, 3 H), 2.15-2.22 (m, 2 H), 4.12 (br d, *J*= 18 Hz, 1 H), 4.36 (br d, *J*= 18 Hz, 1 H), 5.25-5.40 (m, 2 H), 5.56 (s, 2 H), 7.30 (s, 1 H), 7.74 (ddd, J_1= 8 Hz, J_2= 7 Hz, J_3= 1 Hz, 1 H), 7.89 (ddd, J_1= 8 Hz, J_2= 7 Hz, J_3= 1 Hz, 1 H), 8.15 (d, *J*= 1 Hz, 1 H), 8.17 (d, *J*= 1 Hz, 1 H), 8.34-8.48 (br m, 2 H), 8.74 (s, 1 H); ; LRMS (MALDI) *m/z* 428 (M+Na), 406 (M+H), 379, 338, 331, 294, 228, 212, 190, 172, 164..

Example 4



Name: 3-*tert*-Butoxycarbonylamino-propionic acid 4-ethyl-3,13-dioxo-3,4,12,13-tetrahydro-1*H*-2-oxa-6,12a-diaza-dibenzo[*b*,*h*]fluoren-4-yl ester **(4)**

Molecular Weight: 519 g/mol

Molecular Formula: $C_{28}H_{29}N_3O_7$

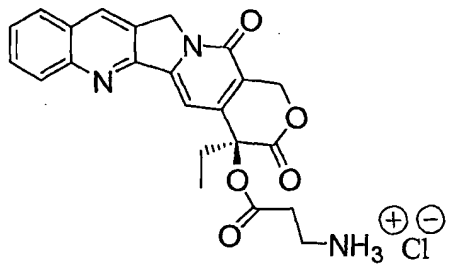
Calculated Log P: 2.6824

Procedure:

Camptothecin(CPT) (0.50 g, 1.4 mmol) was placed in an oven dried flask under Nitrogen. Next anhydrous DMF (12.5 ml) was added followed by 3-*tert*-Butoxycarbonylamino-propionic acid (0.55 g, 2.9 mmol), DMAP (0.087 g, 0.71 mmol) and DCC (0.60 g, 2.9 mmol) generating a yellow slurry. After 5h at 22 °C the reaction became clear and the DMF was concentrated without heating under high vacuum. Next dichloromethane (25x3 ml) was added and the mixture was filtered each time. Finally, the crude material was purified by flash chromatography (99:1 CH_2Cl_2/acetone; 98:2 CH_2Cl_2/acetone; 96:4 CH_2Cl_2/acetone; 9:1 CH_2Cl_2/acetone) to provide **(4)** 306 mg (41% yield) as a light yellow solid: ^{1}H NMR (CD_2Cl_2) 400 MHz δ 0.99 (t, *J*= 7 Hz, 3 H), 1.35 (s, 9 H), 2.00-2.70 (m, 2 H), 2.60-2.80 (m, 2 H), 3.30-3.50 (m, 2 H) 5.10-5.23 (br s, 1 H), 5.26 (br s, 2 H), 5.37 (d, *J*= 17 Hz, 1 H), 5.64 (d, *J*= 17 Hz, 1 H), 7.20 (s, 1 H), 7.67 (ddd, J_1= 8 Hz, J_2= 6 Hz, J_3= 1 Hz, 1 H), 7.83 (ddd, J_1= 8.4 Hz, J_2= 7 Hz, J_3= 2 Hz), 7.97 (d, *J*= 8 Hz, 1 H), 8.17 (d, *J*= 8 Hz, 1 H), 8.41 (s, 1 H) ^{13}C NMR (CD_2Cl_2) 100 MHz δ 8.0, 28.6, 32.0, 35.3, 37.0, 50.5,

67.6, 76.9, 79.5, 96.1, 120.1, 128.4, 128.8, 129.3, 130.0, 131.1, 131.8, 146.4, 147.0, 149.3, 153.0, 156.2, 157.8, 168.3, 172.0; LRMS (EI) *m/z* 519 (M+), 402, 330, 302, 287.

Example 5



Name: 2-(4-Ethyl-3,13-dioxo-3,4,12,13-tetrahydro-1*H*-2-oxa-6,12a-diaza-dibenzo[*b*,*h*]-fluoren-4-yloxycarbonyl)-ethyl-ammonium chloride **(5)**

Molecular Weight: 456 g/mol

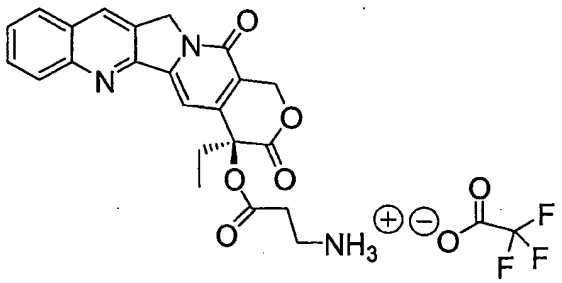
Molecular Formula: $C_{23}H_{22}ClN_3O_5$

Calculated Log P(w/o counterion): 0.9040

Camptothecin ester **(4)** (50 mg, 0.10 mmol) was placed in an oven dried flask under nitrogen and hydrogen chloride in dioxane (10 ml, 4.0 M) was added generating a bright yellow solution. After 5 h the dioxane was concentrated, the residue was washed with ether 3x2 ml and the bright yellow

product was placed under high vacuum overnight providing 43.7 mg (96% yield) of **(5)** as the hydrochloride salt: ^{1}H NMR (d_6-DMSO) 400 MHz δ 0.92 (t, *J*= 7 Hz, 3 H), 2.14-2.23 (m, 2 H), 2.94-3.06 (m, 4 H), 5.26-5.38 (m, 2 H), 5.49 (d, *J*= 17 Hz, 1 H), 5.54 (d, *J*= 17 Hz, 1 H), 7.17 (s, 1 H), 7.73 (ddd, J_1= 8 Hz, J_2= 7 Hz, J_3= 1 Hz, 1 H), 7.88 (ddd, J_1= 8 Hz, J_2= 7 Hz, J_3= 1 Hz, 1 H), 7.92-8.08 (br m, 2 H), 8.15 (d, *J*= 8 Hz, 1 H), 8.17 (d, 8 Hz, 1 H), 8.72 (s, 1 H); HRMS (MALDI) *m/z* Calcd for $C_{23}H_{21}N_3O_5K$ (M+K) 458.111, found 458.111.

Example 6



Name: 2-(4-Ethyl-3,13-dioxo-3,4,12,13-tetrahydro-1*H*-2-oxa-6,12a-diaza-dibenzo[*b*,*h*]-fluoren-4-yloxycarbonyl)-ethyl-ammonium trifluoroacetate **(6)**

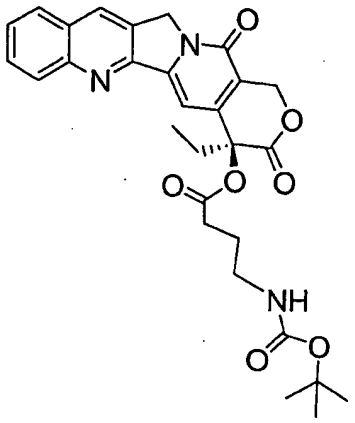
Molecular Weight: 533

Molecular Formula: $C_{25}H_{22}F_3N_3O_7$

Calculated Log P(w/o counterion): 0.9040

Camptothecin ester **(4)** (44.5 mg, 0.09 mmol) was placed in an oven dried flask under nitrogen and anhydrous CH_2Cl_2 (1.0 ml) was added. Next TFA (1.0 ml) was added dropwise at 0 °C. After 5 h at 22 °C the reaction mixture was concentrated providing a light brown oily residue. The residue was washed with ether (3x1 ml) and placed under high vacuum overnight providing **(6)** as a bright yellow solid weighing 35.2 mg (73% yield): ^{1}H NMR (d_6-DMSO) 400 MHz δ 0.92 (t, *J*= 7 Hz, 3 H), 2.10-2.26 (m, 2 H), 2.84-3.10 (m, 4 H), 5.29 (d, *J*= 20 Hz, 1 H), 5.34 (d, *J*= 20 Hz, 1 H), 5.49(d, *J*= 17 Hz, 1 H), 5.54 (d, *J*= 17 Hz, 1 H), 7.17 (s, 1 H), 7.73 (t, *J*= 8 Hz, 1 H), 7.78-7.98 (m, 4 H), 8.15 (d, *J*= 7 Hz, 1 H), 8.17 (d, *J*= 8 Hz, 1 H), 8.72 (s, 1 H); ^{13}C NMR (CD_2Cl_2) 100 MHz δ 7.6, 30.3, 31.1, 34.3, 50.3, 66.5, 76.5, 95.0, 118.9, 127.8, 128.0, 128.7, 128.9, 129.8, 130.6, 131.7, 145.0, 146.1, 147.9, 152.4, 156.5, 167.2, 169.7; LRMS (MALDI) *m/z* 458 (M+K), 442 (M+Na), 420 (M+H), 331, 228, 212, 164.

Example 7



Name: 4-*tert*-Butoxycarbonylamino-butyric acid 4-ethyl-3,13-dioxo-3,4,12,13-tetrahydro-1*H*-2-oxa-6,12a-diaza-dibenzo[*b*,*h*]fluoren-4-yl ester **(7)**

Molecular Weight: 533 g/mol

Molecular Formula: $C_{29}H_{31}N_3O_7$

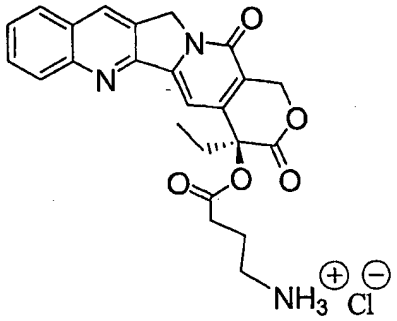
Calculated Log P: 3.0296

Procedure:

Camptothecin(CPT) (0.50 g, 1.4 mmol) was placed in an oven dried flask under Nitrogen. Next anhydrous DMF (12.5 ml) was added followed by 4-*tert*-Butoxycarbonylamino-butyric acid (0.59 g, 2.9 mmol), DMAP (0.087 g, 0.71 mmol) and DCC (0.60 g, 2.9 mmol) generating a

yellow slurry. After 5h at 22 °C the reaction became clear and the DMF was concentrated without heating under high vacuum. Next dichloromethane (25x3 ml) was added and the mixture was filtered each time. Finally, the crude material was purified by flash chromatography (99:1 CH_2Cl_2/acetone; 98:2 CH_2Cl_2/acetone; 96:4 CH_2Cl_2/acetone; 9:1 CH_2Cl_2/acetone) to provide **(7)** 151 mg (20% yield) as a light yellow solid: ^{1}H NMR (CD_2Cl_2) 400 MHz δ 0.97 (t, *J*= 7 Hz, 3 H), 1.39 (s, 9 H), 1.82 (p, *J*= 7 Hz, 2 H), 2.06-2.25 (m, 2 H), 2.52 (t, *J*= 7 Hz, 2 H), 3.00-3.19 (m, 2 H) 4.94-5.02 (br s, 1 H), 5.19 (d, *J*= 20 Hz, 1 H), 5.24 (d, *J*= 20 Hz, 1 H), 5.34 (d, *J*= 17 Hz, 1 H), 5.60 (d, J= 17 Hz, 1 H), 7.15 (s, 1 H), 7.64 (t, *J*= 7 Hz, 1 H), 7.80 (t, *J*= 7 Hz, 1 H), 7.94 (d, *J*= 8 Hz, 1 H), 8.18 (d, *J*= 8 Hz, 1 H), 8.38 (s, 1 H) ^{13}C NMR (CD_2Cl_2) 100 MHz δ 7.9, 25.6, 28.6, 31.5, 32.0, 40.1, 50.5, 67.5, 76.5, 79.3, 96.1, 120.3, 128.4, 128.4, 128.8, 129.3, 129.9, 131.0, 131.8, 146.5, 146.9, 149.3, 153.0, 156.4, 157.7, 168.1, 172.7; LRMS (EI) *m/z* 533 (M+), 477, 348, 330, 302, 287, 248, 218.

Example 8



Name: 3-(4-Ethyl-3,13-dioxo-3,4,12,13-tetrahydro-1*H*-2-oxa-6,12a-diaza-dibenzo[*b*,*h*]-fluoren-4-yloxycarbonyl)-propyl-ammonium chloride **(8)**

Molecular Weight: 470 g/mol

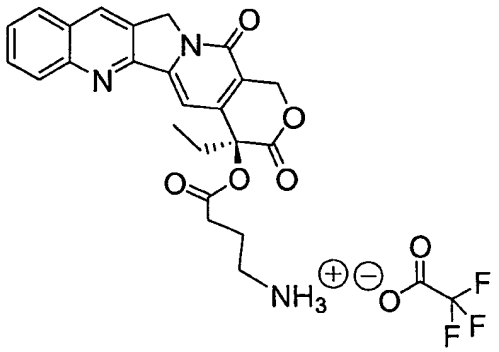
Molecular Formula: $C_{24}H_{24}ClN_3O_5$

Calculated Log P w/o counterion: 1.2686

Camptothecin ester **(7)** (57 mg, 0.11 mmol) was placed in an oven-dried flask under nitrogen and hydrogen chloride in dioxane (4 ml, 4.0 M) was added generating a bright yellow solution. After 6 h the dioxane was concentrated, the residue was washed with ether 3x2 ml and the bright yellow product was placed under high vacuum overnight providing **(8)** 48 mg (93% yield) as the hydrochloride salt: ^{1}H NMR (d_6-DMSO) 400 MHz δ 0.89 (t, *J*= 7 Hz, 3 H), 1.74-1.83 (m, 2 H), 2.06-2.16 (m, 2 H), 2.66 (t, *J*= 7 Hz, 2 H), 2.74-2.84 (m, 2 H), 5.28 (s, 2 H), 5.42-5.52 (m, 2 H), 7.04 (s, 1 H), 7.66-7.72 (m, 1

H), 7.80-7.88 (m, 2), 8.11 (d, *J*= 9 Hz, 1 H), 8.13 (d, *J*= 9 Hz, 1 H), 8.68 (s, 1 H); ^{13}C NMR (CD_2Cl_2) 100 MHz δ 7.6, 22.2, 30.2, 30.3, 37.7, 50.3, 66.4, 76.0, 94.8, 118.9, 127.9, 128.1, 128.7, 128.9, 129.9, 130.6, 131.8, 145.4, 146.1, 147.9, 152.4, 156.6, 167.4, 171.5; LRMS (MALDI) *m/z* 472 (M+K), 456 (M+Na), 434 (M+H).

Example 9



Name: 3-(4-Ethyl-3,13-dioxo-3,4,12,13-tetrahydro-1*H*-2-oxa-6,12a-diaza-dibenzo[*b*,*h*]-fluoren-4-yloxycarbonyl)-propyl-ammonium trifluoro-acetate **(9)**

Molecular Weight: 547 g/mol

Molecular Formula: $C_{26}H_{24}F_3N_3O_7$

Calculated Log P(w/o counterion): 1.2686

Camptothecin ester (7) (38.4 mg, 0.07 mmol) was placed in an oven-dried flask under nitrogen and anhydrous CH_2Cl_2 (1.0 ml) was added. Next TFA (1.0 ml) was added dropwise at 0 °C. After 5 h at 22 °C the reaction mixture was concentrated providing a light brown oily residue. The residue was washed with ether (3x1 ml) and placed under high vacuum overnight providing (9) as a bright yellow solid weighing 32.5 mg (85% yield): ^{1}H NMR (d_6-DMSO) 400 MHz δ 0.93 (t, *J*= 7 Hz, 3 H), 1.75-1.85 (m, 2 H), 2.10-2.20 (m, 2 H), 2.70 (t, *J*= 7 Hz, 2 H), 2.80-2.90 (br m, 2 H), 5.26-5.38 (m, 2 H), 5.46-5.56 (m, 2 H), 7.07 (s, 1 H), 7.68-7.82 (m, 3 H), 7.88 (ddd, J_1= 8 Hz, J_2= 7 Hz, J_3= 2 Hz, 1 H), 8.15 (d, *J*= 8 Hz, 1 H), 8.17 (d, *J*= 8 Hz, 1 H) 8.72 (s, 1 H);^{13}C NMR (CD_2Cl_2) 100 MHz δ 7.6, 22.3, 30.1, 30.2, 37.9, 50.3, 66.3, 76.0, 94.7, 118.8, 127.8, 128.1, 128.6, 128.9, 129.9, 130.5, 131.7, 145.3, 146.1, 147.9, 152.3, 156.6, 167.4, 171.5; HRMS (MALDI) *m/z* Calcd for $C_{24}H_{24}N_3O_5Na$(M+Na) 456.1530, found 456.1552.

Example 10

A HPLC chromatogram depicting the separation of camptothecin (retention time of 6.25 min) from camptothecin carboxylate (retention time of 2.2 min) is shown in Figure 7. Camptothecin samples in PBS buffer were prepared by adding 1 μM camptothecin from a DMSO stock solution. Note at a very brief incubation time of 1 min. camptothecin in its lactone form predominates, but at longer incubation times on the order of several hours the

drug has hydrolyzed extensively and its inactive, ring-opened carboxylate form predominates (right panel). Separation of the lactone and carboxylate forms of camptothecin was achieved using an isocratic mobile phase consisting of a mixture of 41% acetonitrile and 59% of the triethylamine acetate buffer. Both camptothecin forms were detected at an excitation wavelength of 380 nm and an emission wavelength of 440 nm. A flow rate of 1 mL/min was employed.

A HPLC chromatogram depicting the markedly improved solution stability of camptothecin-20-acetate (retention time of 7.1 min) relative to camptothecin (Figure 7) is shown in Figure 8. Note how the camptothecin-20-acetate sample is stable relative to camptothecin; unlike the parent agent, the camptothecin-20-acetate analog does not readily convert to a rapidly eluting carboxylate species upon standing at 37 °C for 3 hr (right panel). Separation of camptothecin-20-acetate was achieved using an isocratic mobile phase consisting of a mixture of 41% acetonitrile to 59% of the triethylamine acetate buffer. Camptothecin-20-acetate was detected at an excitation wavelength of 380 nm and an emission wavelength of 440 nm. A flow rate of 1 mL/min were employed.

Example 11

The improved human blood stability of camptothecin-20-acetate relative to camptothecin in phosphate buffered saline and in human blood

(left panel, 1 μM drug concentration) is shown in Figure 9. While camptothecin-20-acetate was very stable in PBS and blood with only slight instability of the agent in the latter matrix being noted at the longer incubation times, the parent camptothecin agent hydrolyzed rapidly in both PBS and in human blood. Markedly enhanced human blood stability relative to camptothecin was also noted for another ester, camptothecin-20-octanoate (right panel, 1 μM drug concentration). Stability profiles were determined using HPLC methods. All experiments were conducted at pH 7.4 and 37 °C.

Example 12

HPLC chromatograms depicting the high purity and high stability in non-aqueous DMSO solution of a hydrochloride salt preparation (left panel) and a trifluoroacetate salt preparation (right panel) of camptothecin-20-glycinate ester are shown in Figure 10. Upon standing in DMSO for hours the two camptothecin-20-glycinate ester salt forms did not show significant evidence of hydrolysis or other forms of chemical reactivity. Separation of camptothecin-20-glycinate ester was achieved using an isocratic mobile phase consisting of a mixture of 41% acetonitrile to 59% of the triethylamine acetate buffer. Camptothecin-20-glycinate ester was detected at an excitation wavelength of 380 nm and an emission wavelength of 440 nm. A flow rate of 1 mL/min was employed.

Example 13

HPLC chromatograms depicting the pronounced and instantaneous reactivity of camptothecin-20-glycinate ester hydrochloride upon addition to PBS buffer under near physiological conditions of ionic strength and temperature are shown in Figure 11. The parent camptothecin-20-glycinate ester peak appears at a retention time of 2.7 min. Immediately following the addition of camptothecin-20-glycinate ester hydrochloride to PBS buffer at a concentration of 1 μM, a new peak is observed (retention time of 5.2 min). Upon further standing, sampling of the drug solution in PBS shows further decomposition with a total of at least three other chemical entities being observed in the solution. Separation of starting material (camptothecin-20-glycinate ester) from the hydrolysis products (camptothecin-20-lactam intermediate (Retention time of 5.2 min), camptothecin (retention time of 6.3 min) and camptothecin carboxylate (retention time of 2.1 min) was achieved using an isocratic mobile phase consisting of a mixture of 41% acetonitrile to 59% of the triethylamine acetate buffer. Camptothecin-20-glycinate ester and its decomposition products were detected at an excitation wavelength of 380 nm and an emission wavelength of 440 nm. A flow rate of 1 mL/min was employed.

Example 14

HPLC chromatograms depicting the pronounced and instantaneous reactivity of camptothecin-20-glycinate ester hydrochloride upon addition to human blood are provided in Figure 12. Data are presented for samples analyzed almost immediately following the addition of 1 μM camptothecin-20-glycinate ester to human blood (1 minute of incubation, left panel) and at a longer time of incubation of 3 hr (right panel). The parent camptothecin-20-glycinate ester peak appears at a retention time of 3.1 min. Immediately following the addition of camptothecin-20-glycinate ester hydrochloride to blood at a concentration of 1 μM a new peak was observed (retention time of 6.1 min). Upon further standing to a total incubation time of 3 hours in blood, HPLC analysis of the sample showed evidence of essentially complete decomposition. A total of at least three new chemical entities were observed in the blood suspension to which essentially pure camptothecin-20-glycinate ester was added. Separation of starting material (camptothecin-20-glycinate ester) from the reactants [camptothecin-20-lactam intermediate (retention time of 6.1 min); camptothecin (lactone form, retention time of 7.9 min); and camptothecin carboxylate (retention time of 3.0 min)] was achieved using an isocratic mobile phase consisting of a mixture of 41% acetonitrile to 59% of the triethylamine acetate buffer. Camptothecin-20-glycinate ester and its decomposition products were detected at an excitation wavelength

of 380 nm and an emission wavelength of 440 nm. A flow rate of 1 mL/min was employed in the analysis.

Example 15

The pronounced reactivity of camptothecin-20-glycinate ester in human plasma (left panel) and human blood (right panel) at an initial pro-drug concentration of 1 μM is shown in Figure 13. Comparison of the stability of camptothecin-20-glycinate ester in human plasma versus human blood samples reveals that the agent is initially more reactive in the human blood sample. In both samples the levels of reaction intermediate camptothecin-20-lactam were the greatest for the first 30 minutes, and then during the next 90 minute interval of incubation the carboxylate species became predominant. Stability profiles were determined using HPLC methods. All experiments were conducted at 37 °C and sample pH values were adjusted to 7.4 prior to the initiation of an experiment.

Example 16

Depiction of the impact of pH on the chemical decomposition of camptothecin-20-glycinate ester in PBS buffer at 37 °C at a pro-drug concentration of 1 μM are shown in Figure 14. Camptothecin-20-glycinate ester levels were measured using HPLC analysis. While camptothecin-20-glycinate ester was relatively stable at a pH value of 3 (with only slight reactivity observed and 95% of the agent remaining in its original form at an

incubation time of 3 hr.), significant decomposition of the agent occurred at higher pH values. As observed in Figure 11, pronounced and instantaneous reactivity of camptothecin-20-glycinate ester hydrochloride occurred upon addition to PBS buffer at pH 7.4 (solid circles).

Example 17

The effect of pH on the reversibility of the formation of the chemical degradation products of camptothecin-20-glycinate ester in PBS buffer at 37 °C at a pro-drug concentration of 1 μM is shown in Figure 15. Camptothecin-20-glycinate ester levels and decomposition product levels were measured using HPLC analysis. As shown in Figure 11, pronounced and instantaneous reactivity of camptothecin-20-glycinate ester hydrochloride upon addition to PBS buffer at pH 7.4. Solid triangles represent the levels of camptothecin-20-lactam intermediate that had formed while open circles represent the levels of the parent camptothecin-20-glycinate ester pro-drug. Upon reduction of solution pH to a low value of 2, the data shows that camptothecin-20-lactam intermediate levels decrease while camptothecin-20-glycinate ester pro-drug levels become elevated. This data shows that the lactam intermediate can reform camptothecin-20-glycinate ester and hence the reaction is reversible. The formation of the camptothecin-20-glycinate ester upon pH reduction is consistent with the data contained in Figure 14 indicating that the parent camptothecin-20-glycinate ester pro-drug predominates at

reduced pH values (*i.e.* at low pH the agent is much more stable to reacting and forming the lactam intermediate).

Example 18

Reaction mechanisms which camptothecin-20-glycinate ester hydrochloride undergoes in aqueous solution at 37 °C at a pro-drug concentration of 1 μM are illustrated in Figure 16. We are the first to postulate that camptothecin-20-lactam is formed during glycinate ester degradation. Our novel finding concerning the formation of camptothecin-20-lactam correlates with the rapid formation of camptothecin lactone and carboxylate species (versus the much slower formation of lactone and carboxylate forms released following the hydrolysis of an alkyl ester analog of camptothecin such as camptothecin-20-acetate or camptothecin-20-octanoate).

Stability profiles (pH 7.4, 37°C) of several different camptothecin pro-drug structures in PBS buffer are shown in Figure 17, and demonstrate the improved stability of the pro-drug structures. Similarly, Figure 18 shows the improved stability of camptothecin pro-drug structures in whole blood. Data sets are shown for both free drugs in whole blood as well as core-loaded pro-drugs.

Example 19

The normalized fluorescence emission spectra of 1 μM of camptothecin-20-glycinate ester hydrochloride in PBS buffer at different pH values is shown in Figure 19. Note the strong shifting of the emission spectra to the red region upon increasing solution pH. Experiments were conducted at 37 °C using exciting light of 370 nm.

Example 20

Figure 20 shows the fluorescence excitation and emission spectra of 1 μM of camptothecin-20-glycinate ester hydrochloride in PBS buffer in the presence and absence of 0.1 M dimyristoylphosphatidylcholine (DMPC) or 0.1 M dimyristoylphosphatidlyglycerol (DMPG) small unilamellar liposomes. Experiments were conducted at pH 7.4 and 37°C. Note the strong shifting of the emission spectra to the blue region or shorter wavelengths in the presence of liposomes. This spectral shifting is indicative of drug interaction with the membranes. Experiments were conducted using exciting light of 370 nm. Spectral parameters of the various camptothecins and glycinate ester analogs are summarized in Table 13.

Table 1. Fluorescence spectral parameters for Camptothecin analogues in PBS at pH 7.4

Compound	Ex	Em	Relative Fluorescence
CPT	370	430	1
CPT-20-glycinate	370	430	0.8
CPT-20-glycinate			
CPT-20-butanate			
10, 11-MDO-CPT	384	398	1
10, 11-MDO-CPT-20-glycinate	384	398	1
7-Ethyl-10, 11-MDO-CPT	383	398	0.3
7-Ethyl-10,11-MDO-CPT-20-glycinate	383	398	1
7-Chloromethyl-10, 11-MDO-CPT	387	404	0.3
7-Chloromethyl-10, 11-MDO-CPT-20-glycinate	388	404	0.3
9-Amino-CPT	381	439	0.001
9-Amino-CPT-20-glycinate	381	439	0.002
9-Chloro-10, 11-MDO-CPT	385	399	1
9-Chloro-10, 11-MDO-CPT-20-glycinate	385	399	1

9-Amino-10,11-MDO-CPT	383	428	0.002
9-Amino-10,11-MDO-CPT-20-glycinate	383	428	0.002
10-Amino-CPT	397	527	0.4
10-Amino-CPT-20-glycinate	397	527	0.3

Example 21

Fluorescence anisotropy titration was used to study the associations of camptothecin-20-glycinate ester to small unilamellar vesicles composed of electroneutral DMPC (Panel A) and DMPG (Panel B) suspended in phosphate buffered saline. Results are shown in Figure 21. Experiments were conducted at pro-drug concentrations of 1 μM at 37 °C using exciting light of 370 nm. Note that in the presence of increasing amounts of liposomes the drug anisotropy values increase until the majority of drug is liposome-bound. Data is also presented in the two panels for camptothecin for comparative purposes. Analysis of the data reveals that, in the case of DMPC bilayers, camptothecin-20-glycinate has reduced membrane binding associations relative to camptothecin both at pH 7.4 and at pH 3.0 (Panel A, inset). For DMPG bilayers, camptothecin-20-glycinate ester displays markedly higher affinities due to the electrostatic attractive forces between

the positively charged drug and negatively charged membrane. Membrane association constants for various camptothecins and their glycinate esters are summarized in Table 2.

Table 2. Association constants for Camptothecin analogues interacting with unilamellar vesicles of electroneutral DMPC, negatively charged DMPG in PBS at pH 7.4 and 37°C.

Compound	K_{DMPC}	K_{DMPG}
CPT	100	100
CPT-20-glycinate Hydrochloride	≤59	400
CPT-20-glycinate Hydrochloride	≤53	870
CPT-20-butanate Hydrochloride	≤51	1020
10, 11-MDO-CPT	110	100
10, 11-MDO-CPT-20-glycinate Hydrochloride	93	345

7-Ethyl-10, 11-MDO-CPT	160	130
7-Ethyl-10, 11-MDO-CPT-20-glycinate Hydrochloride	220	1161
7-Chloromethyl-10, 11-MDO-CPT	180(3)	160(2)
7-Chloromethyl-10, 11-MDO-CPT-20-glycinate Hydrochloride	93	310
9-Amino-CPT	low fluorescence	
9-Amino-CPT-20-glycinate Hydrochloride	low fluorescence	
9-Chloro-10, 11-MDO-CPT	310	360

9-Chloro-10, 11-MDO-CPT-20-glycinate Hydrochloride	210	1006
9-Amino-10, 11-MDO-CPT	low fluorescence	
9-Amino-10, 11-MDO-CPT-20-glycinate Hydrochloride	low fluorescence	
10-Amino-CPT	37	26
10-Amino-CPT-20-glycinate Hydrochloride	70	36

Example 22

Reactivity of camptothecin-20-glycinate ester in the presence of varying concentrations of DMPC SUVs in PBS buffer at 37 °C at pH 7.4 at an initial pro-drug concentration of 1 μM is shown in Figure 22. Comparison of the stability of camptothecin-20-glycinate ester in the presence of DMPC versus the absence of DMPC reveals that the presence of membrane initially helps conserve the camptothecin-20 glycinate. Our novel findings indicate that following several minutes of incubation, the

presence of membrane has the net effect of markedly promoting the conversion of camptothecin-20-glycinate to camptothecin-20-lactam. The effect of the membranes on promoting levels of camptothecin-20-lactam is dependent upon the concentration of membrane. Stability profiles were determined using HPLC methods.

Example 23

Reactivity of camptothecin-20-glycinate ester in the presence of varying concentrations of DMPC SUVs in PBS buffer at 37 °C at pH 10 (top panel) and pH 3.0 at an initial pro-drug concentration of 1 μM is shown in Figure 23. Comparison of the stability of camptothecin-20-glycinate ester in the presence of DMPC versus the absence of DMPC reveals the presence of membrane initially helps conserve the camptothecin-20 glycinate. Several minutes later the presence of membrane has the net effect of markedly promoting the conversion of camptothecin-20-glycinate to camptothecin-20-lactam. The effect of the membranes on promoting levels of camptothecin-20-lactam is dependent upon the concentration of membrane. Stability profiles were determined using HPLC methods.

Example 24

Stabilization in PBS (left panel) and whole human blood (right panel) by core loading of camptothecin-20-glycinate ester hydrochloride into DSPC liposomes has on pro-drug stability at 37 °C at pH 7.4 is shown

in Figure 24. Comparison of the stability profiles for camptothecin-20-glycinate ester in this figure with data shown in Figure 22 clearly demonstrate that core loading is much more effective at preventing the decomposition of camptothecin-20-glycinate ester relative to bilayer loading. Stability profiles were determined using HPLC methods.

Example 25

Stabilization in PBS (left panel) and whole human blood (right panel) by core loading of camptothecin-20-glycinate ester trifluoroacetate into DSPC liposomes has on pro-drug stability at 37 °C at pH 7.4 is shown in Figure 25. Comparison of the stability profiles for camptothecin-20-glycinate ester with other data again indicate core loading is much more effective at preventing the decomposition of camptothecin-20-glycinate ester relative to bilayer loading. Stability profiles were determined using HPLC methods.

Example 26

Model membrane associations of several A-ring and A,B-ring modified camptothecin glycinate ester compounds to small unilamellar vesicles (SUVs) composed of electroneutral dimyristoylphosphatidylcholine (DMPC) and negatively-charged dimyristoylphosphatidylglycerol (DMPG) in PBS are shown in Figure 26. Data are also shown for camptothecin-20-glycinate ester, 7-chloromethyl-10,11-methylenedioxy-camptothecin-20-

glycinate ester, 9-chloro-10,11-methylenedioxycampto-thecin-20-glycinate ester, and 7-ethyl-10,11-methylenedioxycamptothecin-20-glycinate ester binding to DMPC and DMPG bilayers. The method of fluorescence anisotropy titration was used to construct the adsorption isotherms. Experiments were conducted at drug concentrations of 1 μM in PBS buffer (37 °C). Note how the anisotropy values vary at a given lipid concentration base upon drug structure, with certain A-ring and A,B-ring modifications resulting in significantly more lipophilic agents. Because of the potential for chemical reactivity of the agents of interest in PBS at pH 7.4, anisotropy values at each lipid concentration were determined immediately (approx. 30 sec. to 1 min.) following the addition of the glycinate ester form of each agent to the liposome so as to minimize loss of the parent glycinate ester prior to sample measurement. Table 2 summarizes the K values for DMPC as well as DMPG bilayers.

Example 27

Equilibrium binding of several E-ring modified camptothecin-20-OR esters (where R=$CO[CH_2]_n$ NH_2HCl where n= 1-3) to small unilamellar vesicles (SUVs) composed of electroneutral dimyristoylphosphatidylcholine (DMPC) and negatively-charged dimyristoylphosphatidylglycerol (DMPG) in PBS is shown in Figure 27. Data are also shown for camptothecin-20-glycinate ester, camptothecin-20-propanate ester, and camptothecin-20-

butanate ester. Data for camptothecin is included for comparative purposes. The method of fluorescence anisotropy titration was used to construct the adsorption isotherms. Experiments were conducted at drug concentrations of 1 μM in PBS buffer (37 °C). Because of the potential for chemical reactivity of the agents of interest in PBS at pH 7.4, anisotropy values at each lipid concentration were determined immediately (approx. 30 sec.) following the addition of the glycinate ester form of each agent to the liposome so as to minimize loss of the parent ester prior to sample measurement. Table 2 summarizes the K values for DMPC as well as DMPG bilayers.

Example 28

Reactivity of highly lipophilic 7-t-Butyldimethylsilyl-10-hydroxycamptothecin glycinate ester (DB-67 glycinate) in PBS buffer (left panel) and human blood (right panel) at an initial pro-drug concentration of 1 μM is shown in Figure 28. In both samples the levels of reaction intermediate camptothecin-20-lactam were the greatest for the first 30 minutes, and the following 90 minutes of incubation the carboxylate species became predominant. Stability profiles were determined using HPLC methods. All experiments were conducted at 37 °C and sample pH values were adjusted to 7.4 prior to the initiation of an experiment.

Example 29

Reactivities of several E-ring modified camptothecin-20-OR esters (having the formula R=CO[CH_2]$_n$$NH_2$HCl where n= 1-3) in PBS buffer (left panel) and human blood (right panel) at an initial pro-drug concentration of 1 μM are shown in Figure 29. Note the novel finding depicting how the stabilities of the compounds increase with increasing n value. The following order of chemical stability was observed for the agents of interest in both PBS and whole human blood: camptothecin-20-glycinate ester >> camptothecin-20-propanate ester >> camptothecin-20-butanate ester. In the case of the propanate and butanate ester samples no lactam peak was observed by HPLC chromatographic analysis indicating the 7-membered and 8-membered lactam species are far less stable in either PBS solution or blood suspension relative to the camptothecin-20-lactam (which can be readily separated), during HPLC chromatographic analysis. Stability profiles were determined using HPLC methods. All experiments were conducted at 37 °C and sample pH values were adjusted to 7.4 prior to the initiation of an experiment.

Example 30

Reactivity of camptothecin-20-propanate ester hydrochloride in the presence of varying concentrations of DMPC SUVs in PBS buffer at 37 °C at pH 7.4 at an initial pro-drug concentration of 1 μM is shown in Figure 30. In dramatic contrast to situation observed for camptothecin-20-glycinate

where the presence of lipid bilayer can promote the decomposition of the the agent, the presence of DMPC clearly aids in stabilizing the camptothecin-20-OR ester (where $R=CO[CH_2]_nNH_2HCl$ where n=1 for glycinate and n=2 for propanate). The stabilization effect by DMPC on the camptothecin-20-propanate is dependent upon the concentration of the lipid where greater hydrolysis is observed at the lower lipid concentrations. Stability profiles were determined using HPLC methods.

Example 31

Reactivity of camptothecin-20-butanate ester hydrochloride in the presence of varying concentrations of DMPC SUVs in PBS buffer at 37 °C at pH 7.4 at an initial pro-drug concentration of 1 μM is shown in Figure 31. As observed in the case of camptothecin-20-propanate and in dramatic contrast to the situation observed for camptothecin-20-glycinate (where lipid bilayer interactions can promote the decomposition of the agent), the presence of DMPC aids in stabilizing the camptothecin-20-OR ester (where $R=CO[CH_2]_nNH_2HCl$ and n=1 for glycinate and n=3 for butanate). The stabilization effect by DMPC on the camptothecin-20-butanate, as observed in the case of camptothecin-20-propanate, is dependent upon the concentration of the lipid where greater hydrolysis is observed at the lower lipid concentrations. Stability profiles were determined using HPLC methods.

Example 32

Improved stability of core-loaded liposomal camptothecin-20-propanate in PBS buffer relative to the corresponding data set for camptothecin-20-glycinate is shown in Figure 32. Note at times as long as 3 hrs that liposomal camptothecin-20-propanate remains greater than 98% intact and unreacted (Panel A), while at times out to 48 hrs greater than 35% of the liposomal formulation remains as it original pro-drug form (Panel B). Stability profiles were determined using HPLC methods. All experiments were conducted at an original pro-drug concentration of 1 μM. Similarly, improved stability of liposomal core-loaded camptothecin-20-propanate ester in PBS versus bilayer-loaded is shown in Figure 33.

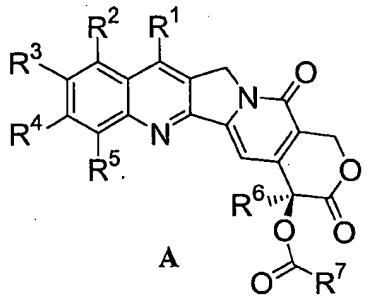
Example 33

Improved stability of core-loaded liposomal camptothecin-20-butanate pro-drug dispersed in human blood versus drug in its free unencapsulated form is shown in Figure 34. Note at times as long as 3 hrs that liposomal camptothecin-20-butanate remains greater than 98% intact and unreacted (Panel A). Similarly, Figures 35-38 show improved stability of core-loaded liposomal DB-67 glycinate (Fig. 35), free DB-67 butanate (Fig. 36), core-loaded liposomal camptothecin-20-glycinate ester (Fig. 37) and liposomal DB-67 butanate ester (Fig. 38) in blood and PBS.

The foregoing description of preferred embodiments of the invention has been presented for purposes of illustration and description. It is not intended to be exhaustive or to limit the invention to the precise form disclosed. Obvious modifications or variations are possible in light of the above teachings. The embodiments were chosen and described to provide the best illustration of the principles of the invention and its practical application to thereby enable one of ordinary skill in the art to utilize the invention in various embodiments and with various modifications as are suited to the particular use contemplated. All such modifications and variations are within the scope of the invention as determined by the appended claims when interpreted in accordance with the breadth to which they are fairly, legally and equitably entitled.

What is claimed is:

1. A composition comprising a camptothecin-20-aminoester derivative having the structure:



A

A) wherein R^1 is:

1) hydrogen, a halogen atom, a branched or linear alkyl group, a branched or linear alkenyl group, a C_{3-7} cycloalkyl group, a branched or linear alkynyl group, an alkoxy group, an alkylamino group, a dialkylamino group, an alkylthiol group, a thiol group, a phenyl group, an amino group, a nitro group, or a cyano group; or

2) $(CH_2)_YNR_8R_9$, wherein: (a) Y is an integer from 1-10 and R_8 and R_9 are, independently, hydrogen, an alkyl group, an alkenyl

group , an alkynyl group, an amine, an alkyl amine, a dialkyl amine, a hydroxy group, an alkoxy group, an acyl group, or a carbamate, and (b) R_8, R_9 and the nitrogen to which they are attached may form a saturated or unsaturated three- to ten-membered heterocyclic ring containing O, S, and NR^{10} wherein R^{10} is a hydrogen, an alkyl group, an alkenyl group, an alkynyl group, an alkoxy group or a carbamate; or

3) a C_{1-10} cycloalkyl group, a C_{1-10} cycloalkenyl group, or a C_{1-10} cycloalkynyl group;

B) wherein R^2 is:

1) hydrogen, a halogen atom, a linear or branched alkyl group, a linear or branched alkenyl group, a linear or branched alkynyl group, an amino group, an alkylamino group, a dialkylamino group, a nitro group, a 3-10 membered heterocyclic ring, a C_{3-10} cycloalkyl group, a C_{3-10} cycloalkenyl group, a C_{3-10} cycloalkynyl group, a thiol group, or a cyano group;

2) $(CH_2)_YNR_8R_9$, wherein: (a) Y is an integer from 1-10; and (b) R_8 and R_9 are, independently, hydrogen, an alkyl group, an alkenyl group , an alkynyl group, an amine, an alkyl amine, a dialkyl amine, a hydroxy group, an alkoxy group, an acyl group, or a carbamate; and (c) R_8, R_9 and the nitrogen to which they are attached may form a saturated or unsaturated three to ten membered heterocyclic ring;

C) wherein R^3 is:

1) hydrogen, a halogen atom, a linear or branched alkyl group, a linear or branched alkenyl group, a linear or branched alkynyl group, an amino group, an alkylamino group, a dialkylamino group, a nitro group, a 3-10 membered heterocyclic ring, a C_{3-10} cycloalkyl group, a C_{3-10} cycloalkenyl group, a C_{3-10} cycloalkynyl group, a thiol group, a cyano group, a hydroxyl group; or

2) $(CH_2)_YNR_8R_9$, wherein: (a) Y is an integer from 1-10; (b) R_8 and R_9 are, independently, hydrogen, an alkyl group, an alkenyl group, an alkynyl group, an amine, an alkyl amine, a dialkyl amine, a hydroxy group, an alkoxy group, an acyl group, a carbamate; and (c) R_8, R_9 and the nitrogen to which they are attached may form a saturated or unsaturated three to ten membered heterocyclic ring;

D) wherein R^4 is a hydrogen, a halogen atom, a hydroxy group, an amino group, a methoxy group, an alkyl group, an alkynyl group or an alkenyl group;

E) wherein R^5 is hydrogen or fluorine;

F) wherein R^6 is an alkyl group, an alkenyl group, an alkynyl group, or a benzyl group; and

G) wherein R^7 is:

1) a side chain of a naturally occurring amino acid; or

2) $(CH_2)_LNR^{14}R^{15}$, wherein L is an integer ranging from 1-30 and R^{14} and R^{15} are independently the same or different and are hydrogen, a C_{1-15} alkyl group, a C_{2-15} alkenyl group, a C_{2-15} alkynyl group or an aryl group.

2. The composition of claim 1, wherein R^1 is linked to R^2 in accordance with the structure $R^1(CH_2)_QR^2$, wherein Q represents an integer 1-10, a $SiR_{11}R_{12}R_{13}$ group, or a $(CH_2)_FSiR_{11}R_{12}R_{13}$ group, wherein:

A) F is an integer from 1-10; and

B) R_{11}, R_{12} and R_{13} independently represent hydrogen, a halogen atom, an alkyl group, an alkenyl group, an alkynyl group, a C_{3-10} cycloalkyl group, a C_{3-10} cycloalkenyl group, a C_{3-10} cycloalkynyl group, an amino group, or a hydroxy group.

3. The composition of claim 1, wherein R^2 is linked to R^3 in accordance with the structure $R^2(CH_2)_GR^3$, wherein:

A) G is an integer from 1-10; and

B) one or more N, O or S atoms are substituted for one or more $-CH_2-$ groups.

4. The composition of claim 1, wherein R^3 is linked to R^4 in accordance with the structure $R^3(CH_2)_GR^4$ wherein:

A) G is an integer from 1-10; and

B) one or more N, O or S atoms are substituted for one or more $-CH_2-$ groups.

5. The composition of claim 1, wherein L is an integer ranging from 1-6.

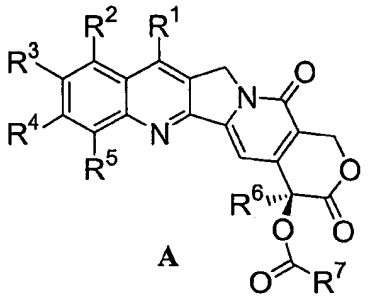
6. The composition of claim 1, wherein said camptothecin-20-aminoester derivative is derived from a camptothecin selected from the group consisting of SN-38, 9-aminocamptothecin, DX-8951f, GG-211, 9-nitrocamptothecin, topotecan, CPT-11, lurtotecan, CKD-602, 10-hydroxycamptothecin, and ST1481.

7. A method for reducing toxicity of a camptothecin, comprising the steps of:

synthesizing a camptothecin-20-aminoester pro-drug; and

incorporating said camptothecin-20-aminoester pro-drug into an aqueous core of a liposome.

8. The method of claim 7, wherein said camptothecin-20-aminoester pro-drug is a camptothecin derivative having the structure:



A

A) wherein R^1 is:

1) hydrogen, a halogen atom, a branched or linear alkyl group, a branched or linear alkenyl group, a C_{3-7} cycloalkyl group, a branched or linear alkynyl group, an alkoxy group, an alkylamino group, a dialkylamino group, an alkylthiol group, a thiol group, a phenyl group, an amino group, a nitro group, or a cyano group; or

2) $(CH_2)_YNR_8R_9$, wherein: (a) Y is an integer from 1-10 and R_8 and R_9 are, independently, hydrogen, an alkyl group, an alkenyl group , an alkynyl group, an amine, an alkyl amine, a dialkyl amine, a hydroxy group, an alkoxy group, an acyl group, or a carbamate, and (b) R_8,

R_9 and the nitrogen to which they are attached may form a saturated or unsaturated three- to ten-membered heterocyclic ring containing O, S, and NR^{10} wherein R^{10} is a hydrogen, an alkyl group, an alkenyl group, an alkynyl group, an alkoxy group or a carbamate; or

3) a C_{1-10} cycloalkyl group, a C_{1-10} cycloalkenyl group, or a C_{1-10} cycloalkynyl group;

B) wherein R^2 is:

1) hydrogen, a halogen atom, a linear or branched alkyl group, a linear or branched alkenyl group, a linear or branched alkynyl group, an amino group, an alkylamino group, a dialkylamino group, a nitro group, a 3-10 membered heterocyclic ring, a C_{3-10} cycloalkyl group, a C_{3-10} cycloalkenyl group, a C_{3-10} cycloalkynyl group, a thiol group, or a cyano group;

2) $(CH_2)_YNR_8R_9$, wherein: (a) Y is an integer from 1-10; and (b) R_8 and R_9 are, independently, hydrogen, an alkyl group, an alkenyl group , an alkynyl group, an amine, an alkyl amine, a dialkyl amine, a hydroxy group, an alkoxy group, an acyl group, or a carbamate; and (c) R_8, R_9 and the nitrogen to which they are attached may form a saturated or unsaturated three to ten membered heterocyclic ring;

C) wherein R^3 is:

1) hydrogen, a halogen atom, a linear or branched alkyl group, a linear or branched alkenyl group, a linear or branched alkynyl group, an amino group, an alkylamino group, a dialkylamino group, a nitro group, a 3-10 membered heterocyclic ring, a C_{3-10} cycloalkyl group, a C_{3-10} cycloalkenyl group, a C_{3-10} cycloalkynyl group, a thiol group, a cyano group, a hydroxyl group; or

2) $(CH_2)_YNR_8R_9$, wherein: (a) Y is an integer from 1-10; (b) R_8 and R_9 are, independently, hydrogen, an alkyl group, an alkenyl group, an alkynyl group, an amine, an alkyl amine, a dialkyl amine, a hydroxy group, an alkoxy group, an acyl group, a carbamate; and (c) R_8, R_9 and the nitrogen to which they are attached may form a saturated or unsaturated three to ten membered heterocyclic ring;

D) wherein R^4 is a hydrogen, a halogen atom, a hydroxy group, an amino group, a methoxy group, an alkyl group, an alkynyl group or an alkenyl group;

E) wherein R^5 is hydrogen or fluorine;

F) wherein R^6 is an alkyl group, an alkenyl group, an alkynyl group, or a benzyl group; and

G) wherein R^7 is:

1) a side chain of a naturally occurring amino acid; or

2) $(CH_2)_LNR^{14}R^{15}$, wherein L is an integer ranging from 1-30 and R^{14} and R^{15} are independently the same or different and are hydrogen, a C_{1-15} alkyl group, a C_{2-15} alkenyl group, a C_{2-15} alkynyl group or an aryl group.

9. The method of claim 8, wherein R^1 is linked to R^2 in accordance with the structure $R^1(CH_2)_QR^2$, wherein Q represents an integer 1-10, a $SiR_{11}R_{12}R_{13}$ group, or a $(CH_2)_FSiR_{11}R_{12}R_{13}$ group, wherein:

A) F is an integer from 1-10; and

B) R_{11}, R_{12} and R_{13} independently represent hydrogen, a halogen atom, an alkyl group, an alkenyl group, an alkynyl group, a C_{3-10} cycloalkyl group, a C_{3-10} cycloalkenyl group, a C_{3-10} cycloalkynyl group, an amino group, or a hydroxy group.

10. The method of claim 8, wherein R^2 is linked to R^3 in accordance with the structure $R^2(CH_2)_GR^3$, wherein:

A) G is an integer from 1-10; and

B) one or more N, O or S atoms are substituted for one or more $-CH_2-$ groups.

11. The method of claim 8, wherein R^3 is linked to R^4 in accordance with the structure $R^3(CH_2)_GR^4$ wherein:

A) G is an integer from 1-10; and

B) one or more N, O or S atoms are substituted for one or more $-CH_2-$ groups.

12. The method of claim 8, wherein L is an integer ranging from 1-6.

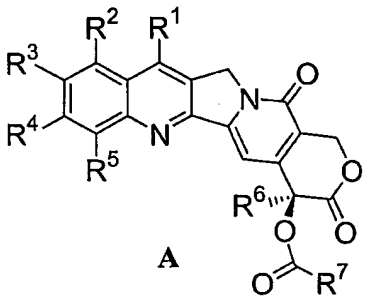
13. The method of claim 8, wherein said camptothecin-20-aminoester derivative is derived from a camptothecin selected from the group consisting of SN-38, 9-aminocamptothecin, DX-8951f, GG-211, 9-nitrocamptothecin, topotecan, CPT-11, lurtotecan, CKD-602, 10-hydroxycamptothecin, and ST1481.

14. A method for extending in vivo survival of a camptothecin comprising the steps of:

synthesizing a camptothecin-20-aminoester pro-drug; and

incorporating said camptothecin-20-aminoester pro-drug into an aqueous core of a liposome.

15. The method of claim 14, wherein said camptothecin-20-aminoester pro-drug is a camptothecin derivative having the structure:



A

A) wherein R^1 is:

1) hydrogen, a halogen atom, a branched or linear alkyl group, a branched or linear alkenyl group, a C_{3-7} cycloalkyl group, a branched or linear alkynyl group, an alkoxy group, an alkylamino group, a dialkylamino group, an alkylthiol group, a thiol group, a phenyl group, an amino group, a nitro group, or a cyano group; or

2) $(CH_2)_YNR_8R_9$, wherein: (a) Y is an integer from 1-10 and R_8 and R_9 are, independently, hydrogen, an alkyl group, an alkenyl group , an alkynyl group, an amine, an alkyl amine, a dialkyl amine, a hydroxy group, an alkoxy group, an acyl group, or a carbamate, and (b) R_8,

R_9 and the nitrogen to which they are attached may form a saturated or unsaturated three- to ten-membered heterocyclic ring containing O, S, and NR^{10} wherein R^{10} is a hydrogen, an alkyl group, an alkenyl group, an alkynyl group, an alkoxy group or a carbamate; or

3) a C_{1-10} cycloalkyl group, a C_{1-10} cycloalkenyl group, or a C_{1-10} cycloalkynyl group;

B) wherein R^2 is:

1) hydrogen, a halogen atom, a linear or branched alkyl group, a linear or branched alkenyl group, a linear or branched alkynyl group, an amino group, an alkylamino group, a dialkylamino group, a nitro group, a 3-10 membered heterocyclic ring, a C_{3-10} cycloalkyl group, a C_{3-10} cycloalkenyl group, a C_{3-10} cycloalkynyl group, a thiol group, or a cyano group;

2) $(CH_2)_YNR_8R_9$, wherein: (a) Y is an integer from 1-10; and (b) R_8 and R_9 are, independently, hydrogen, an alkyl group, an alkenyl group , an alkynyl group, an amine, an alkyl amine, a dialkyl amine, a hydroxy group, an alkoxy group, an acyl group, or a carbamate; and (c) R_8, R_9 and the nitrogen to which they are attached may form a saturated or unsaturated three to ten membered heterocyclic ring;

C) wherein R^3 is:

1) hydrogen, a halogen atom, a linear or branched alkyl group, a linear or branched alkenyl group, a linear or branched alkynyl group, an amino group, an alkylamino group, a dialkylamino group, a nitro group, a 3-10 membered heterocyclic ring, a C_{3-10} cycloalkyl group, a C_{3-10} cycloalkenyl group, a C_{3-10} cycloalkynyl group, a thiol group, a cyano group, a hydroxyl group; or

2) $(CH_2)_YNR_8R_9$, wherein: (a) Y is an integer from 1-10; (b) R_8 and R_9 are, independently, hydrogen, an alkyl group, an alkenyl group, an alkynyl group, an amine, an alkyl amine, a dialkyl amine, a hydroxy group, an alkoxy group, an acyl group, a carbamate; and (c) R_8, R_9 and the nitrogen to which they are attached may form a saturated or unsaturated three to ten membered heterocyclic ring;

D) wherein R^4 is a hydrogen, a halogen atom, a hydroxy group, an amino group, a methoxy group, an alkyl group, an alkynyl group or an alkenyl group;

E) wherein R^5 is hydrogen or fluorine;

F) wherein R^6 is an alkyl group, an alkenyl group, an alkynyl group, or a benzyl group; and

G) wherein R^7 is:

1) a side chain of a naturally occurring amino acid; or

2) $(CH_2)_LNR^{14}R^{15}$, wherein L is an integer ranging from 1-30 and R^{14} and R^{15} are independently the same or different and are hydrogen, a C_{1-15} alkyl group, a C_{2-15} alkenyl group, a C_{2-15} alkynyl group or an aryl group.

16. The method of claim 15, wherein R^1 is linked to R^2 in accordance with the structure $R^1(CH_2)_QR^2$, wherein Q represents an integer 1-10, a $SiR_{11}R_{12}R_{13}$ group, or a $(CH_2)_FSiR_{11}R_{12}R_{13}$ group, wherein:

A) F is an integer from 1-10; and

B) R_{11}, R_{12} and R_{13} independently represent hydrogen, a halogen atom, an alkyl group, an alkenyl group, an alkynyl group, a C_{3-10} cycloalkyl group, a C_{3-10} cycloalkenyl group, a C_{3-10} cycloalkynyl group, an amino group, or a hydroxy group.

17. The method of claim 15, wherein R^2 is linked to R^3 in accordance with the structure $R^2(CH_2)_GR^3$, wherein:

A) G is an integer from 1-10; and

B) one or more N, O or S atoms are substituted for one or more $-CH_2-$ groups.

18. The method of claim 15, wherein R^3 is linked to R^4 in accordance with the structure $R^3(CH_2)_GR^4$ wherein:

A) G is an integer from 1-10; and

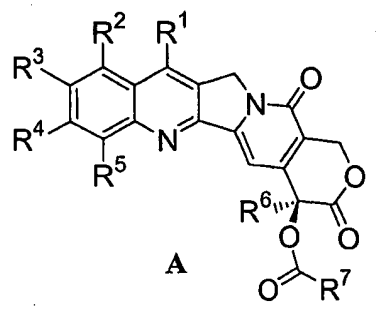
B) one or more N, O or S atoms are substituted for one or more $-CH_2-$ groups.

19. The method of claim 15, wherein L is an integer ranging from 1-6.

20. The method of claim 15, wherein said camptothecin-20-aminoester derivative is derived from a camptothecin selected from the group consisting of SN-38, 9-aminocamptothecin, DX-8951f, GG-211, 9-nitrocamptothecin, topotecan, CPT-11, lurtotecan, CKD-602, 10-hydroxycamptothecin, and ST1481.

21. A pharmaceutical composition comprising an amine-containing camptothecin derivative incorporated into an aqueous core of a liposome.

22. The composition of claim 21, wherein said camptothecin derivative is a camptothecin-20-aminoester having the structure:



A

A) wherein R^1 is:

1) hydrogen, a halogen atom, a branched or linear alkyl group, a branched or linear alkenyl group, a C_{3-7} cycloalkyl group, a branched or linear alkynyl group, an alkoxy group, an alkylamino group, a dialkylamino group, an alkylthiol group, a thiol group, a phenyl group, an amino group, a nitro group, or a cyano group; or

2) $(CH_2)_YNR_8R_9$, wherein: (a) Y is an integer from 1-10 and R_8 and R_9 are, independently, hydrogen, an alkyl group, an alkenyl group , an alkynyl group, an amine, an alkyl amine, a dialkyl amine, a hydroxy group, an alkoxy group, an acyl group, or a carbamate, and (b) R_8, R_9 and the nitrogen to which they are attached may form a saturated or unsaturated three- to ten-membered heterocyclic ring containing O, S, and

NR^{10} wherein R^{10} is a hydrogen, an alkyl group, an alkenyl group, an alkynyl group, an alkoxy group or a carbamate; or

3) a C_{1-10} cycloalkyl group, a C_{1-10} cycloalkenyl group, or a C_{1-10} cycloalkynyl group;

B) wherein R^2 is:

1) hydrogen, a halogen atom, a linear or branched alkyl group, a linear or branched alkenyl group, a linear or branched alkynyl group, an amino group, an alkylamino group, a dialkylamino group, a nitro group, a 3-10 membered heterocyclic ring, a C_{3-10} cycloalkyl group, a C_{3-10} cycloalkenyl group, a C_{3-10} cycloalkynyl group, a thiol group, or a cyano group;

2) $(CH_2)_YNR_8R_9$, wherein: (a) Y is an integer from 1-10; and (b) R_8 and R_9 are, independently, hydrogen, an alkyl group, an alkenyl group , an alkynyl group, an amine, an alkyl amine, a dialkyl amine, a hydroxy group, an alkoxy group, an acyl group, or a carbamate; and (c) R_8, R_9 and the nitrogen to which they are attached may form a saturated or unsaturated three to ten membered heterocyclic ring;

C) wherein R^3 is:

1) hydrogen, a halogen atom, a linear or branched alkyl group, a linear or branched alkenyl group, a linear or branched alkynyl group, an amino group, an alkylamino group, a

dialkylamino group, a nitro group, a 3-10 membered heterocyclic ring, a C_{3-10} cycloalkyl group, a C_{3-10} cycloalkenyl group, a C_{3-10} cycloalkynyl group, a thiol group, a cyano group, a hydroxyl group; or

2) $(CH_2)_YNR_8R_9$, wherein: (a) Y is an integer from 1-10; (b) R_8 and R_9 are, independently, hydrogen, an alkyl group, an alkenyl group, an alkynyl group, an amine, an alkyl amine, a dialkyl amine, a hydroxy group, an alkoxy group, an acyl group, a carbamate; and (c) R_8, R_9 and the nitrogen to which they are attached may form a saturated or unsaturated three to ten membered heterocyclic ring;

D) wherein R^4 is a hydrogen, a halogen atom, a hydroxy group, an amino group, a methoxy group, an alkyl group, an alkynyl group or an alkenyl group;

E) wherein R^5 is hydrogen or fluorine;

F) wherein R^6 is an alkyl group, an alkenyl group, an alkynyl group, or a benzyl group; and

G) wherein R^7 is:

1) a side chain of a naturally occurring amino acid; or

2) $(CH_2)_LNR^{14}R^{15}$, wherein L is an integer ranging from 1-30 and R^{14} and R^{15} are independently the same or different

and are hydrogen, a C_{1-15} alkyl group, a C_{2-15} alkenyl group, a C_{2-15} alkynyl group or an aryl group.

23. The composition of claim 22, wherein R^1 is linked to R^2 in accordance with the structure $R^1(CH_2)_QR^2$, wherein Q represents an integer 1-10, a $SiR_{11}R_{12}R_{13}$ group, or a $(CH_2)_FSiR_{11}R_{12}R_{13}$ group, wherein:

A) F is an integer from 1-10; and

B) R_{11}, R_{12} and R_{13} independently represent hydrogen, a halogen atom, an alkyl group, an alkenyl group, an alkynyl group, a C_{3-10} cycloalkyl group, a C_{3-10} cycloalkenyl group, a C_{3-10} cycloalkynyl group, an amino group, or a hydroxy group.

24. The composition of claim 22, wherein R^2 is linked to R^3 in accordance with the structure $R^2(CH_2)_GR^3$, wherein:

A) G is an integer from 1-10; and

B) one or more N, O or S atoms are substituted for one or more $-CH_2-$ groups.

25. The composition of claim 22, wherein R^3 is linked to R^4 in accordance with the structure $R^3(CH_2)_GR^4$ wherein:

A) G is an integer from 1-10; and

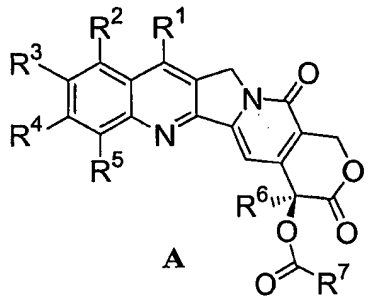
B) one or more N, O or S atoms are substituted for one or more -CH_2- groups.

26. The composition of claim 22, wherein L is an integer ranging from 1-6.

27. The composition of claim 22, wherein said camptothecin-20-aminoester derivative is derived from a camptothecin selected from the group consisting of SN-38, 9-aminocamptothecin, DX-8951f, GG-211, 9-nitrocamptothecin, topotecan, CPT-11, lurtotecan, CKD-602, 10-hydroxycamptothecin, and ST1481.

28. A method of forming a topoisomerase I-inhibiting camptothecin compound in a mammal, comprising administering a liposome preparation comprising a liposome containing a camptothecin-20-aminoester derivative in an aqueous core, said liposome preparation being administered to said mammal in an amount sufficient to inhibit topoisomerase I.

29. The method of claim 28, wherein said camptothecin-20-aminoester derivative has the structure:



A

A) wherein R^1 is:

1) hydrogen, a halogen atom, a branched or linear alkyl group, a branched or linear alkenyl group, a C_{3-7} cycloalkyl group, a branched or linear alkynyl group, an alkoxy group, an alkylamino group, a dialkylamino group, an alkylthiol group, a thiol group, a phenyl group, an amino group, a nitro group, or a cyano group; or

2) $(CH_2)_YNR_8R_9$, wherein: (a) Y is an integer from 1-10 and R_8 and R_9 are, independently, hydrogen, an alkyl group, an alkenyl group , an alkynyl group, an amine, an alkyl amine, a dialkyl amine, a hydroxy group, an alkoxy group, an acyl group, or a carbamate, and (b) R_8, R_9 and the nitrogen to which they are attached may form a saturated or unsaturated three- to ten-membered heterocyclic ring containing O, S, and

NR^{10} wherein R^{10} is a hydrogen, an alkyl group, an alkenyl group, an alkynyl group, an alkoxy group or a carbamate; or

3) a C_{1-10} cycloalkyl group, a C_{1-10} cycloalkenyl group, or a C_{1-10} cycloalkynyl group;

B) wherein R^2 is:

1) hydrogen, a halogen atom, a linear or branched alkyl group, a linear or branched alkenyl group, a linear or branched alkynyl group, an amino group, an alkylamino group, a dialkylamino group, a nitro group, a 3-10 membered heterocyclic ring, a C_{3-10} cycloalkyl group, a C_{3-10} cycloalkenyl group, a C_{3-10} cycloalkynyl group, a thiol group, or a cyano group;

2) $(CH_2)_YNR_8R_9$, wherein: (a) Y is an integer from 1-10; and (b) R_8 and R_9 are, independently, hydrogen, an alkyl group, an alkenyl group , an alkynyl group, an amine, an alkyl amine, a dialkyl amine, a hydroxy group, an alkoxy group, an acyl group, or a carbamate; and (c) R_8, R_9 and the nitrogen to which they are attached may form a saturated or unsaturated three to ten membered heterocyclic ring;

C) wherein R^3 is:

1) hydrogen, a halogen atom, a linear or branched alkyl group, a linear or branched alkenyl group, a linear or branched alkynyl group, an amino group, an alkylamino group, a

dialkylamino group, a nitro group, a 3-10 membered heterocyclic ring, a C_{3-10} cycloalkyl group, a C_{3-10} cycloalkenyl group, a C_{3-10} cycloalkynyl group, a thiol group, a cyano group, a hydroxyl group; or

2) $(CH_2)_YNR_8R_9$, wherein: (a) Y is an integer from 1-10; (b) R_8 and R_9 are, independently, hydrogen, an alkyl group, an alkenyl group, an alkynyl group, an amine, an alkyl amine, a dialkyl amine, a hydroxy group, an alkoxy group, an acyl group, a carbamate; and (c) R_8, R_9 and the nitrogen to which they are attached may form a saturated or unsaturated three to ten membered heterocyclic ring;

D) wherein R^4 is a hydrogen, a halogen atom, a hydroxy group, an amino group, a methoxy group, an alkyl group, an alkynyl group or an alkenyl group;

E) wherein R^5 is hydrogen or fluorine;

F) wherein R^6 is an alkyl group, an alkenyl group, an alkynyl group, or a benzyl group; and

G) wherein R^7 is:

1) a side chain of a naturally occurring amino acid; or

2) $(CH_2)_LNR^{14}R^{15}$, wherein L is an integer ranging from 1-30 and R^{14} and R^{15} are independently the same or different

and are hydrogen, a C_{1-15} alkyl group, a C_{2-15} alkenyl group, a C_{2-15} alkynyl group or an aryl group.

30. The method of claim 29, wherein R^1 is linked to R^2 in accordance with the structure $R^1(CH_2)_QR^2$, wherein Q represents an integer 1-10, a $SiR_{11}R_{12}R_{13}$ group, or a $(CH_2)_FSiR_{11}R_{12}R_{13}$ group, wherein:

A) F is an integer from 1-10; and

B) R_{11}, R_{12} and R_{13} independently represent hydrogen, a halogen atom, an alkyl group, an alkenyl group, an alkynyl group, a C_{3-10} cycloalkyl group, a C_{3-10} cycloalkenyl group, a C_{3-10} cycloalkynyl group, an amino group, or a hydroxy group.

31. The method of claim 29, wherein R^2 is linked to R^3 in accordance with the structure $R^2(CH_2)_GR^3$, wherein:

A) G is an integer from 1-10; and

B) one or more N, O or S atoms are substituted for one or more $-CH_2-$ groups.

32. The method of claim 29, wherein R^3 is linked to R^4 in accordance with the structure $R^3(CH_2)_GR^4$ wherein:

A) G is an integer from 1-10; and

B) one or more N, O or S atoms are substituted for one or more -CH_2- groups.

33. The method of claim 29, wherein L is an integer ranging from 1-6.

34. The method of claim 29, wherein said camptothecin-20-aminoester derivative is derived from a camptothecin selected from the group consisting of SN-38, 9-aminocamptothecin, DX-8951f, GG-211, 9-nitrocamptothecin, topotecan, CPT-11, lurtotecan, CKD-602, 10-hydroxycamptothecin, and ST1481.

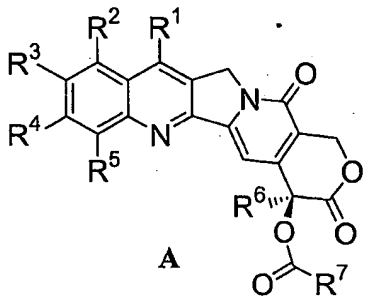
35. The method of claim 28, comprising administering sufficient liposome preparation to provide from about 1 to about 200 mg/kg body weight per week of the camptothecin-20-aminoester derivative.

36. The method of claim 28, wherein said liposome preparation is administered parenterally.

37. A method of treating cancer in a mammal, comprising administering an effective amount of a liposome preparation comprising a

liposome containing a camptothecin-20-aminoester derivative in an aqueous core.

38. The method of claim 37, wherein said camptothecin-20-aminoester derivative has the structure:



A

A) wherein R^1 is:

1) hydrogen, a halogen atom, a branched or linear alkyl group, a branched or linear alkenyl group, a C_{3-7} cycloalkyl group, a branched or linear alkynyl group, an alkoxy group, an alkylamino group, a dialkylamino group, an alkylthiol group, a thiol group, a phenyl group, an amino group, a nitro group, or a cyano group; or

2) $(CH_2)_YNR_8R_9$, wherein: (a) Y is an integer from 1-10 and R_8 and R_9 are, independently, hydrogen, an alkyl group, an alkenyl group , an alkynyl group, an amine, an alkyl amine, a dialkyl amine, a hydroxy group, an alkoxy group, an acyl group, or a carbamate, and (b) R_8, R_9 and the nitrogen to which they are attached may form a saturated or unsaturated three- to ten-membered heterocyclic ring containing O, S, and NR^{10} wherein R^{10} is a hydrogen, an alkyl group, an alkenyl group, an alkynyl group, an alkoxy group or a carbamate; or

3) a C_{1-10} cycloalkyl group, a C_{1-10} cycloalkenyl group, or a C_{1-10} cycloalkynyl group;

B) wherein R^2 is:

1) hydrogen, a halogen atom, a linear or branched alkyl group, a linear or branched alkenyl group, a linear or branched alkynyl group, an amino group, an alkylamino group, a dialkylamino group, a nitro group, a 3-10 membered heterocyclic ring, a C_{3-10} cycloalkyl group, a C_{3-10} cycloalkenyl group, a C_{3-10} cycloalkynyl group, a thiol group, or a cyano group;

2) $(CH_2)_YNR_8R_9$, wherein: (a) Y is an integer from 1-10; and (b) R_8 and R_9 are, independently, hydrogen, an alkyl group, an alkenyl group , an alkynyl group, an amine, an alkyl amine, a dialkyl amine, a hydroxy group, an alkoxy group, an acyl group, or a carbamate;

and (c) R_8, R_9 and the nitrogen to which they are attached may form a saturated or unsaturated three to ten membered heterocyclic ring;

C) wherein R^3 is:

1) hydrogen, a halogen atom, a linear or branched alkyl group, a linear or branched alkenyl group, a linear or branched alkynyl group, an amino group, an alkylamino group, a dialkylamino group, a nitro group, a 3-10 membered heterocyclic ring, a C_{3-10} cycloalkyl group, a C_{3-10} cycloalkenyl group, a C_{3-10} cycloalkynyl group, a thiol group, a cyano group, a hydroxyl group; or

2) $(CH_2)_YNR_8R_9$, wherein: (a) Y is an integer from 1-10; (b) R_8 and R_9 are, independently, hydrogen, an alkyl group, an alkenyl group, an alkynyl group, an amine, an alkyl amine, a dialkyl amine, a hydroxy group, an alkoxy group, an acyl group, a carbamate; and (c) R_8, R_9 and the nitrogen to which they are attached may form a saturated or unsaturated three to ten membered heterocyclic ring;

D) wherein R^4 is a hydrogen, a halogen atom, a hydroxy group, an amino group, a methoxy group, an alkyl group, an alkynyl group or an alkenyl group;

E) wherein R^5 is hydrogen or fluorine;

F) wherein R^6 is an alkyl group, an alkenyl group, an alkynyl group, or a benzyl group; and

G) wherein R^7 is:

1) a side chain of a naturally occurring amino acid; or

2) $(CH_2)_LNR^{14}R^{15}$, wherein L is an integer ranging from 1-30 and R^{14} and R^{15} are independently the same or different and are hydrogen, a C_{1-15} alkyl group, a C_{2-15} alkenyl group, a C_{2-15} alkynyl group or an aryl group.

39. The method of claim 38, wherein R^1 is linked to R^2 in accordance with the structure $R^1(CH_2)_QR^2$, wherein Q represents an integer 1-10, a $SiR_{11}R_{12}R_{13}$ group, or a $(CH_2)_FSiR_{11}R_{12}R_{13}$ group, wherein:

A) F is an integer from 1-10; and

B) R_{11}, R_{12} and R_{13} independently represent hydrogen, a halogen atom, an alkyl group, an alkenyl group, an alkynyl group, a C_{3-10} cycloalkyl group, a C_{3-10} cycloalkenyl group, a C_{3-10} cycloalkynyl group, an amino group, or a hydroxy group.

40. The method of claim 38, wherein R^2 is linked to R^3 in accordance with the structure $R^2(CH_2)_GR^3$, wherein:

A) G is an integer from 1-10; and

B) one or more N, O or S atoms are substituted for one or more -CH_2- groups.

41. The method of claim 38, wherein R^3 is linked to R^4 in accordance with the structure $R^3(CH_2)_GR^4$ wherein:

A) G is an integer from 1-10; and

B) one or more N, O or S atoms are substituted for one or more -CH_2- groups.

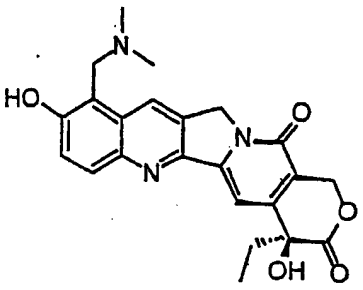
42. The method of claim 38, wherein L is an integer ranging from 1-6.

43. The method of claim 38, wherein said camptothecin-20-aminoester derivative is derived from a camptothecin selected from the group consisting of SN-38, 9-aminocamptothecin, DX-8951f, GG-211, 9-nitrocamptothecin, topotecan, CPT-11, lurtotecan, CKD-602, 10-hydroxycamptothecin, and ST1481.

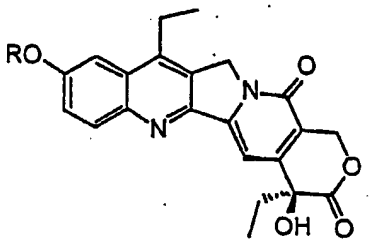
44. The method of claim 37, comprising administering sufficient liposome preparation to provide from about 1 to about 200 mg/kg body weight per week of the camptothecin-20-aminoester derivative.

45. The method of claim 37, wherein said liposome preparation is administered parenterally.

46. The method of claim 37, wherein said mammal is a human.

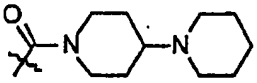


Topotecan/TPT



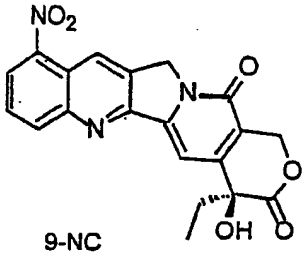
R=H

SN-38

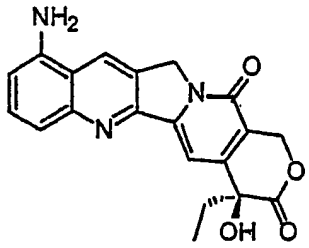
R= 

Irinotecan/CPT-11

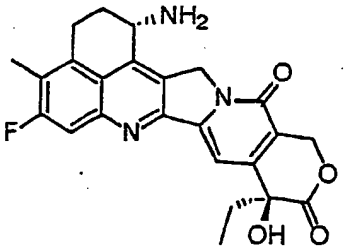
FDA Approved Camptothecins



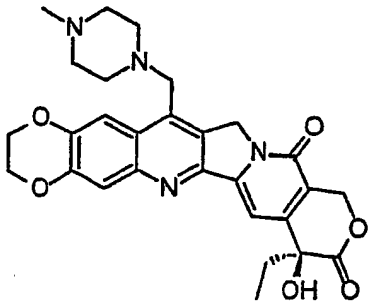
9-NC
Rubitecan



9-AC



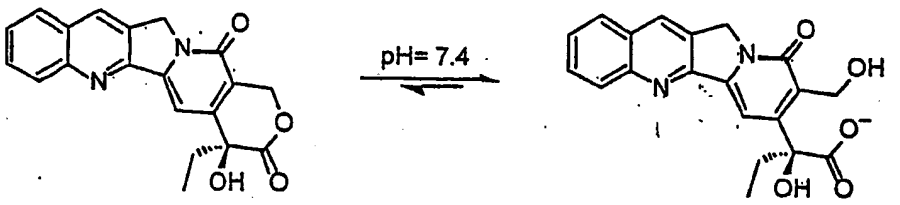
DX-8951f



GI-147211C/GG-211

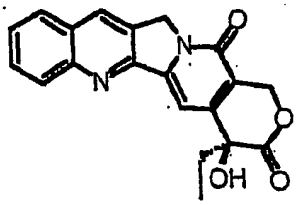
Camptothecins undergoing Clinical Trials

Fig. 1

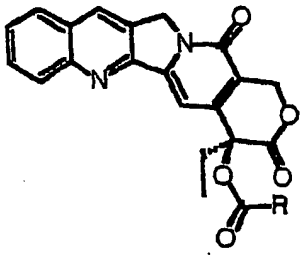


CPT CPT Carboxylate

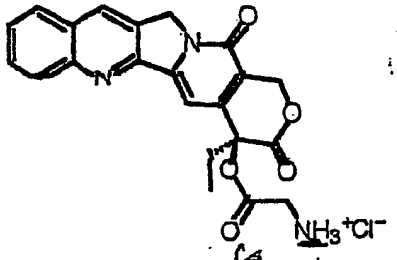
Fig. 2



Camptothecin



R= Linear alkyl chain
i.e. CH_3, C_2H_5 etc.



Camptothecin Glycinate Ester
Hydrochloride

Fig. 3

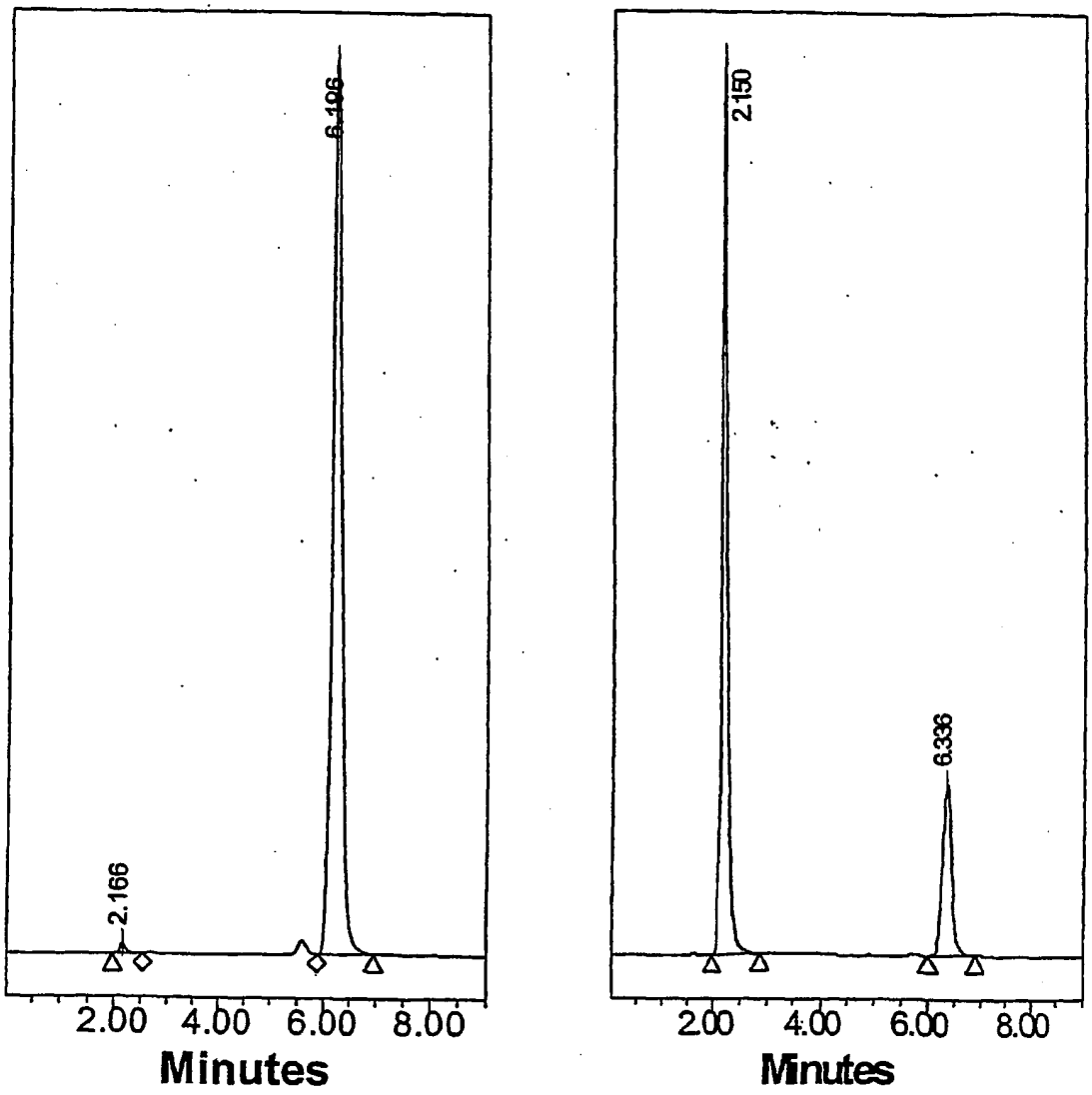

6.196
2.166
2.00
4.00
6.00
8.00
Minutes
2.150
6.336
2.00
4.00
6.00
8.00
Minutes

Fig. 4

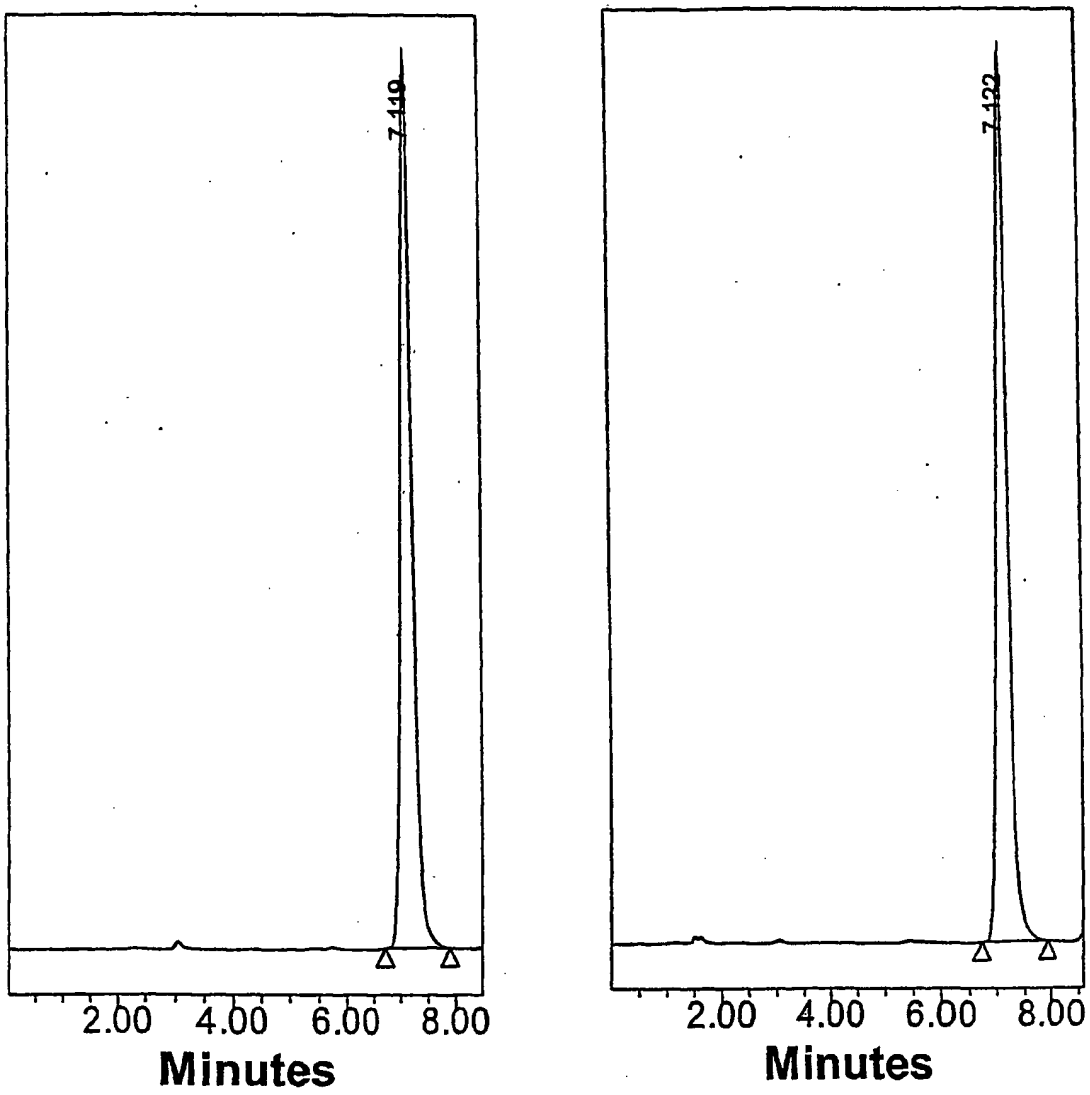


Fig. 5

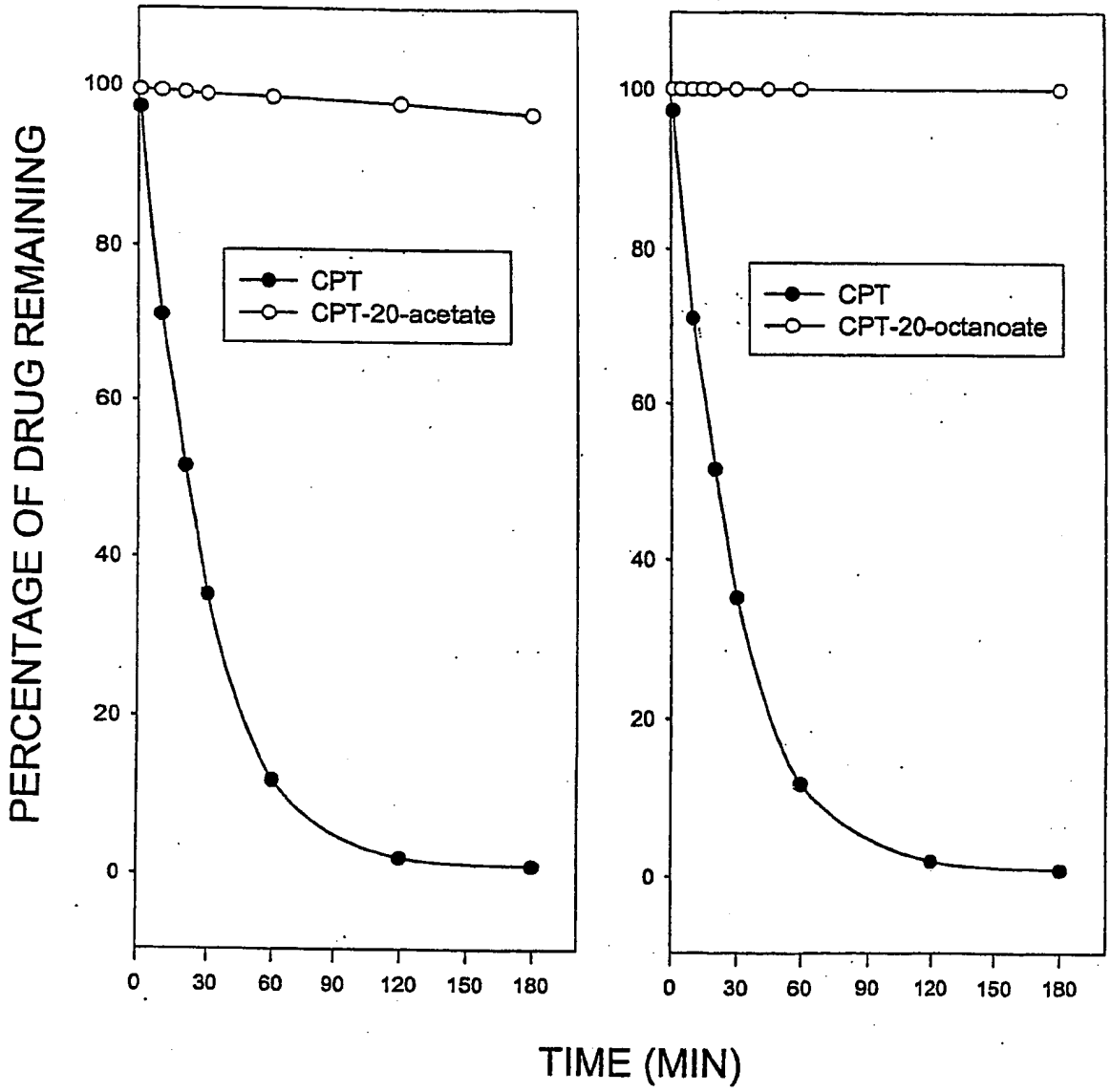
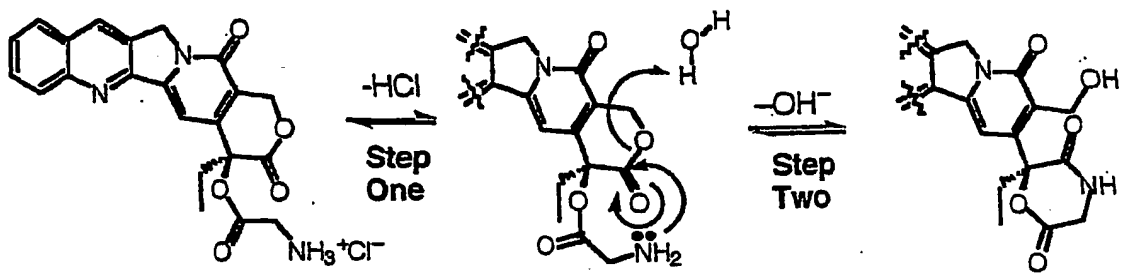

PERCENTAGE OF DRUG REMAINING
100
80
60
40
20
0
CPT
CPT-20-acetate
0 30 60 90 120 150 180
CPT
CPT-20-octanoate
0 30 60 90 120 150 180
TIME (MIN)

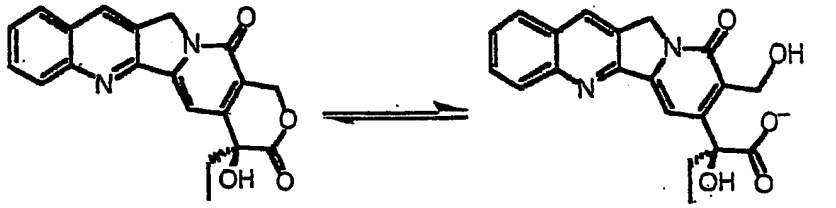
Fig. 6



Camptothecin 20-Glycinate Ester Hydrochloride (1)

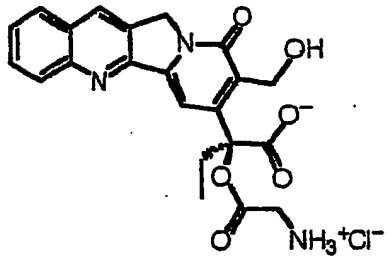
Camptothecin 20-Glycinate Ester Free Base (2)

Lactam Intermediate (3)



Camptothecin Lactone

Camptothecin Carboxylate



Camptothecin Glycinate Carboxylate

Fig. 7

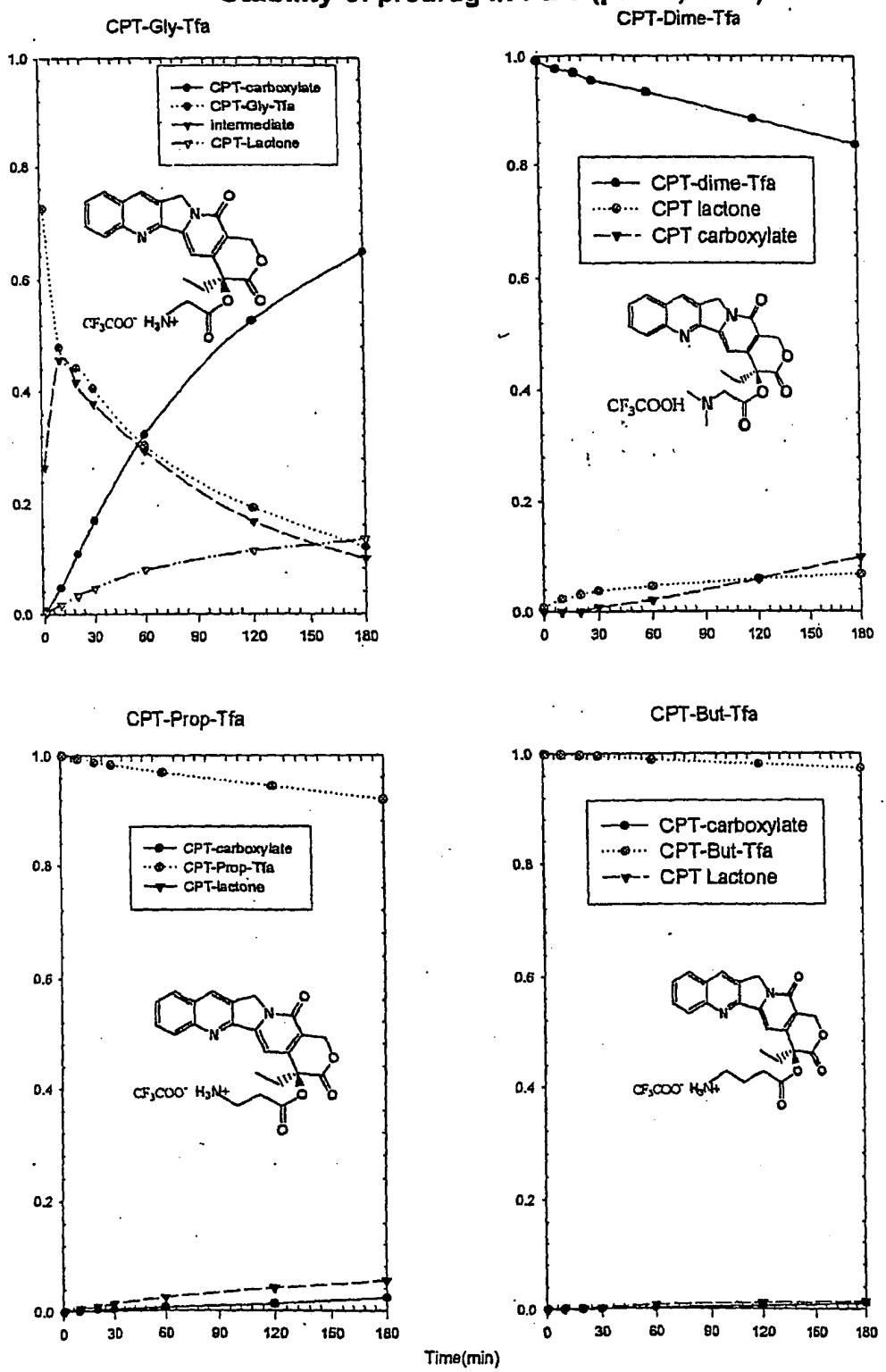
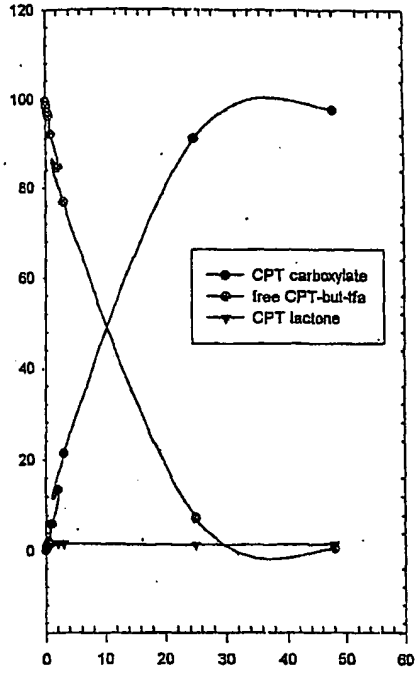
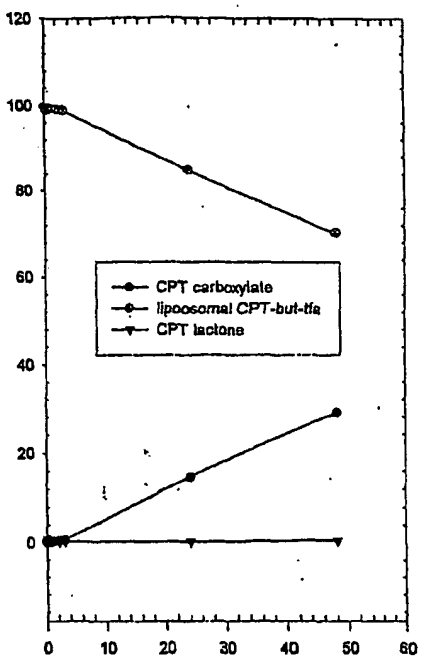

Stability of prodrug in PBS (pH7.4, 37°C)
CPT-Gly-Tfa
CPT-carboxylate
CPT-Gly-Tfa
intermediate
CPT-Lactone
CPT-Dime-Tfa
CPT-dime-Tfa
CPT lactone
CPT carboxylate
CPT-Prop-Tfa
CPT-carboxylate
CPT-Prop-Tfa
CPT-lactone
CPT-But-Tfa
CPT-carboxylate
CPT-But-Tfa
CPT Lactone
Time(min)

Fig. 8

Free CPT-Butanate ester in Whole Blood

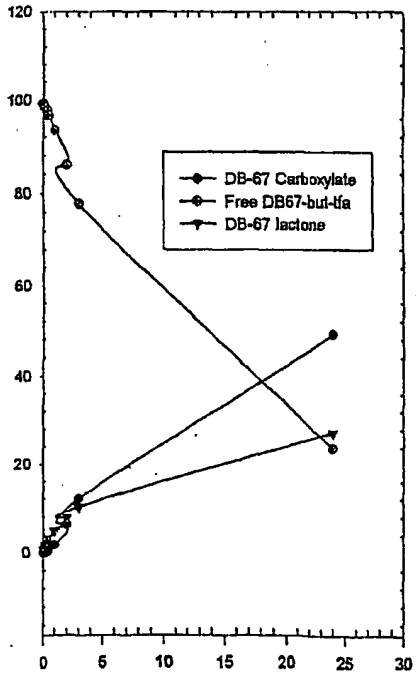


Core-loaded CPT-butanate ester in Whole Blood

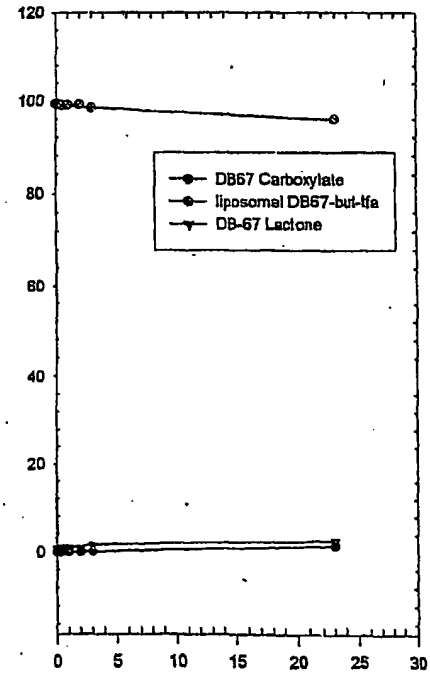


Time (hour)

Free DB-67 butanate in Whole Blood



Core-loaded DB-67 butanate in Whole Blood



Time (hour)

Fig. 9

Normalized Fluorescence spectra of CPT-20-Glycinate in PBS at different pH

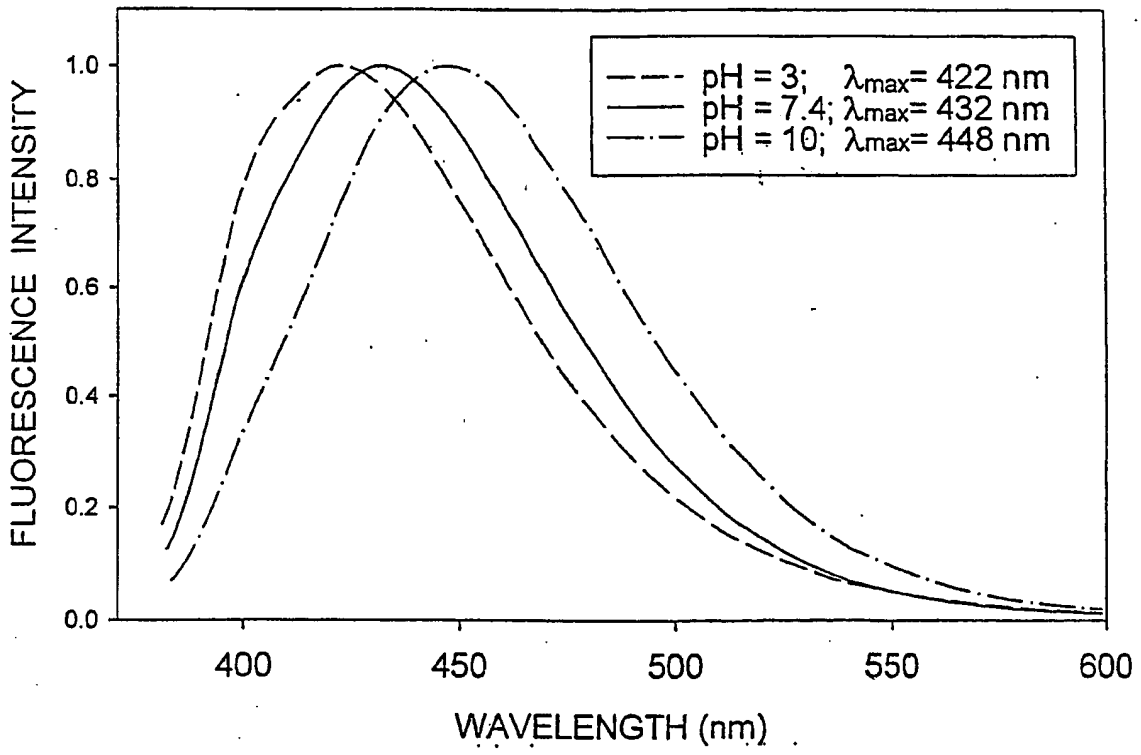


Fig. 10

Excitation and Emission Fluorescence Spectra of CPT-20-Glycinate in PBS and 0.1 M DMPC and DMPG pH=7.4

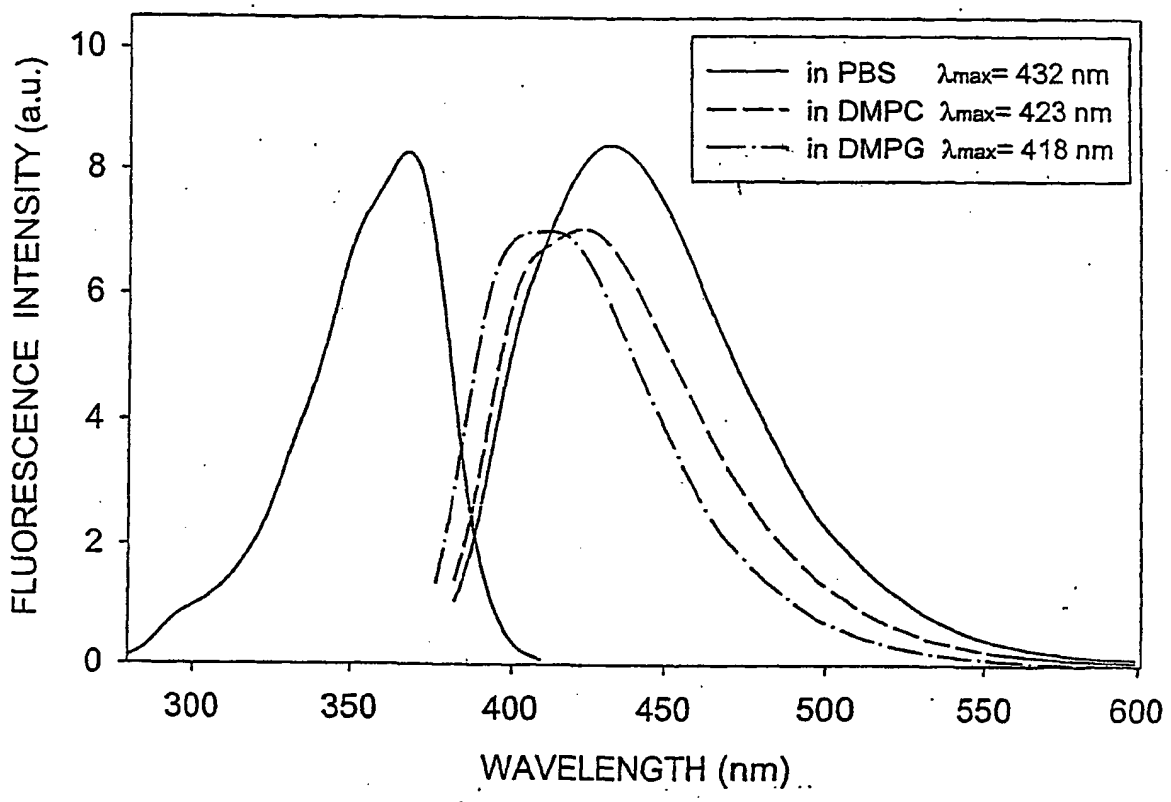


Fig. 11

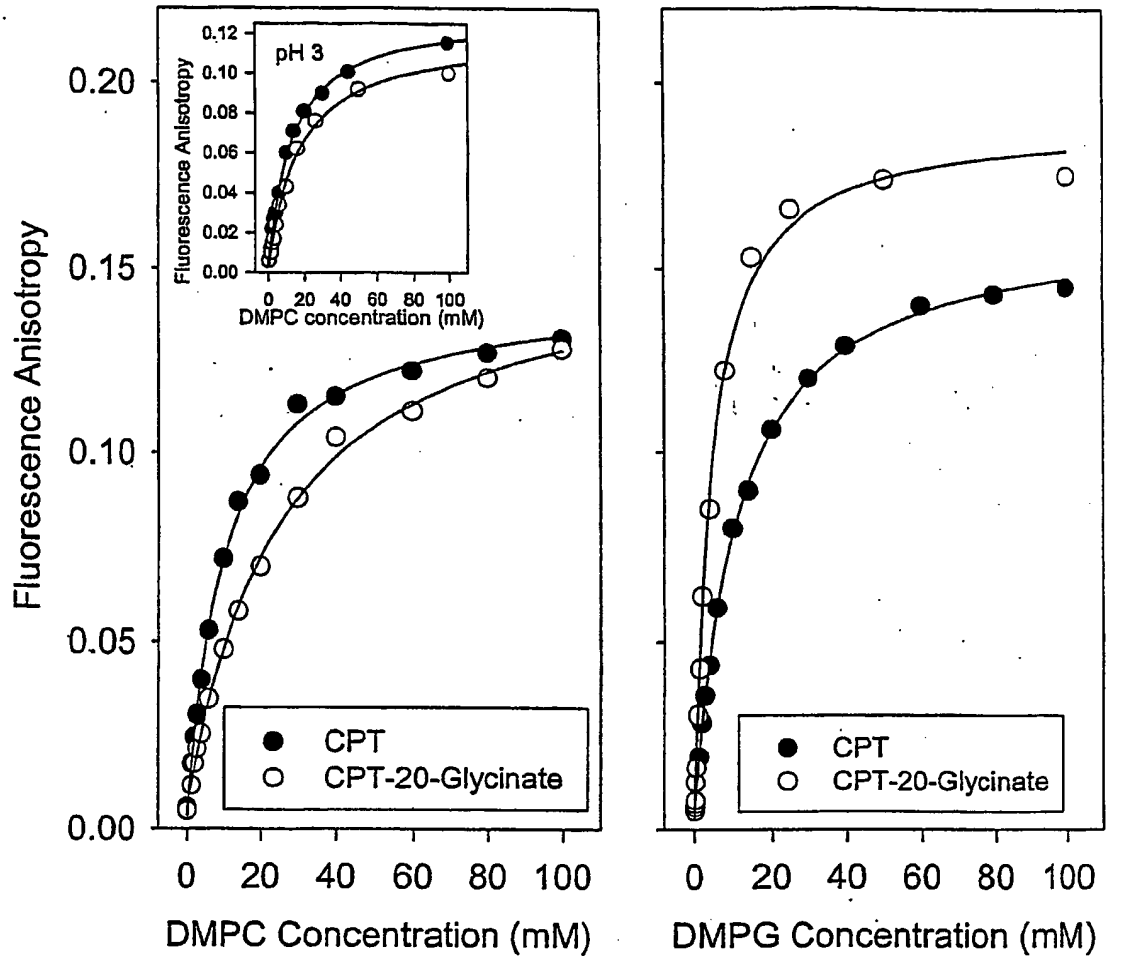
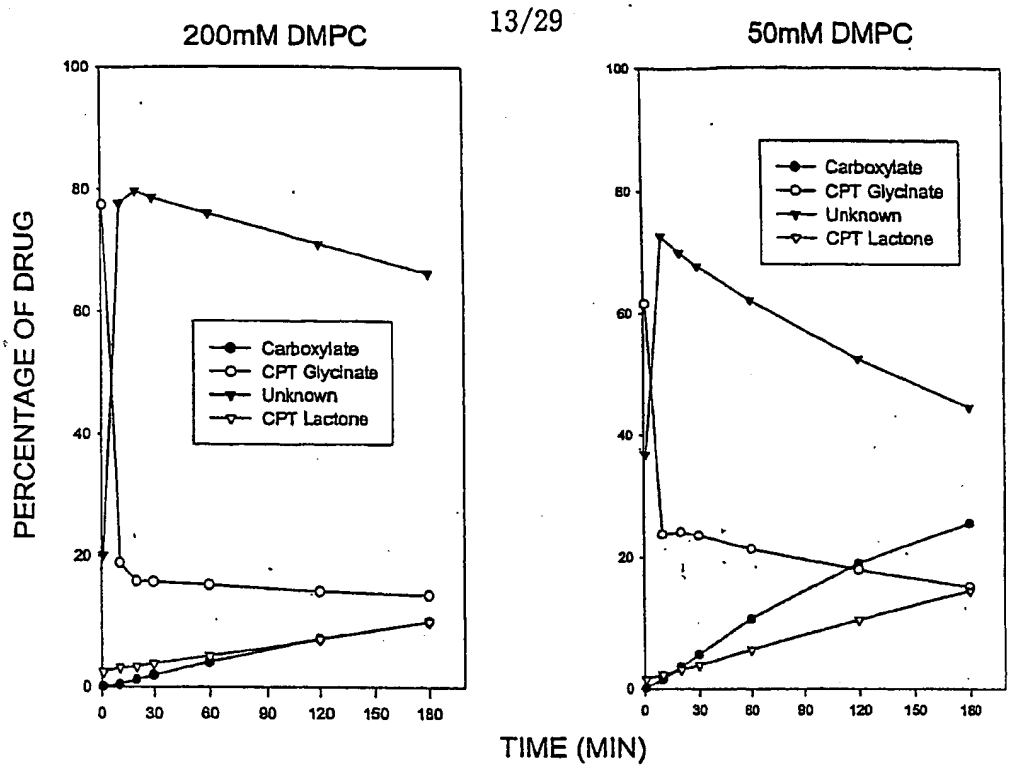

Fluorescence Anisotropy
0.20
0.15
0.10
0.05
0.00
0 20 40 60 80 100
DMPC Concentration (mM)
CPT
CPT-20-Glycinate
pH 3
Fluorescence Anisotropy
0.12
0.10
0.08
0.06
0.04
0.02
0.00
0 20 40 60 80 100
DMPC concentration (mM)
0 20 40 60 80 100
DMPG Concentration (mM)
CPT
CPT-20-Glycinate

Fig. 12


200mM DMPC
50mM DMPC
PERCENTAGE OF DRUG
TIME (MIN)
Carboxylate
CPT Glycinate
Unknown
CPT Lactone
0
20
40
60
80
100
0
30
60
90
120
150
180

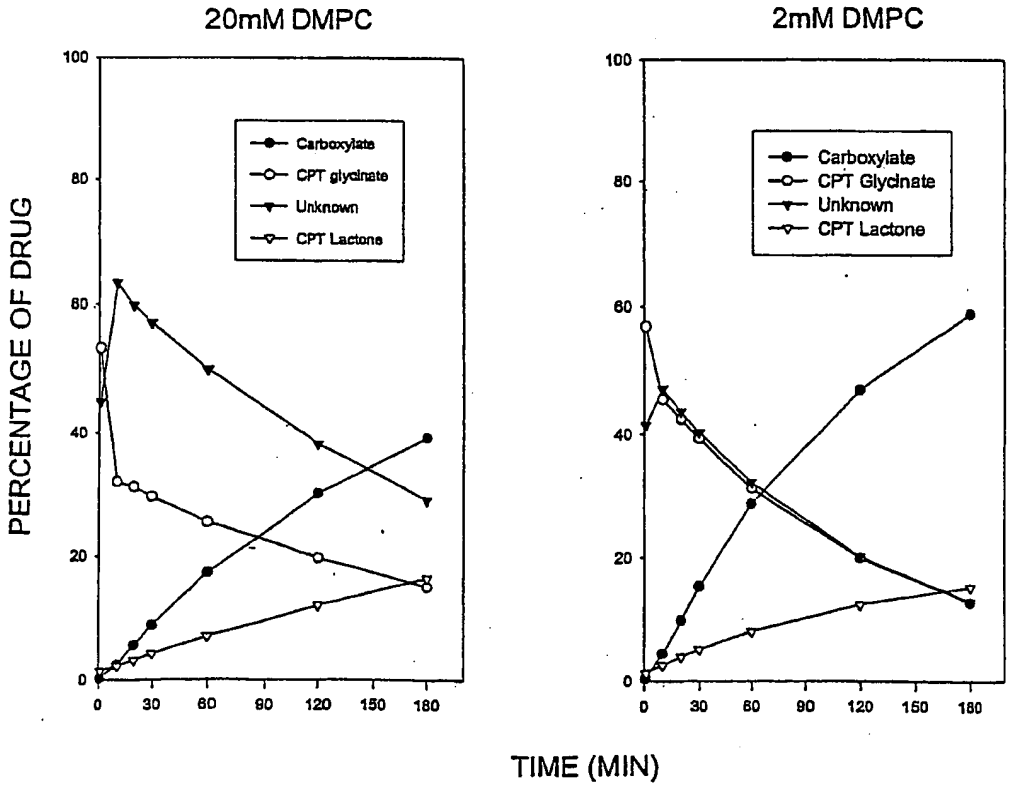

20mM DMPC
2mM DMPC
PERCENTAGE OF DRUG
TIME (MIN)
Carboxylate
CPT glycinate
CPT Glycinate
Unknown
CPT Lactone
0
20
40
60
80
100
0
30
60
90
120
150
180

Fig. 13

Stability of camptothecin -20-glycinate in DMPC at different pH.

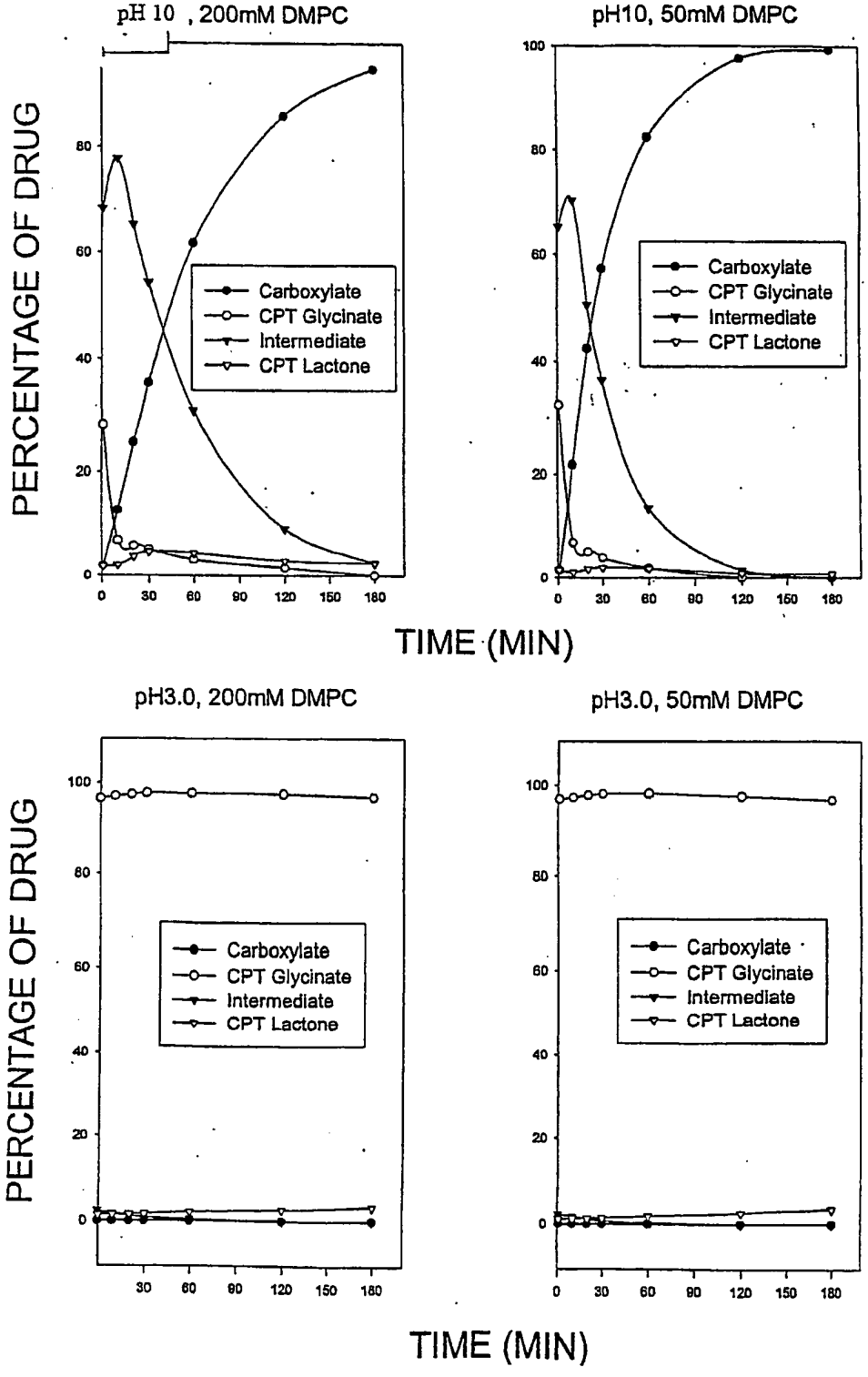


Fig. 14

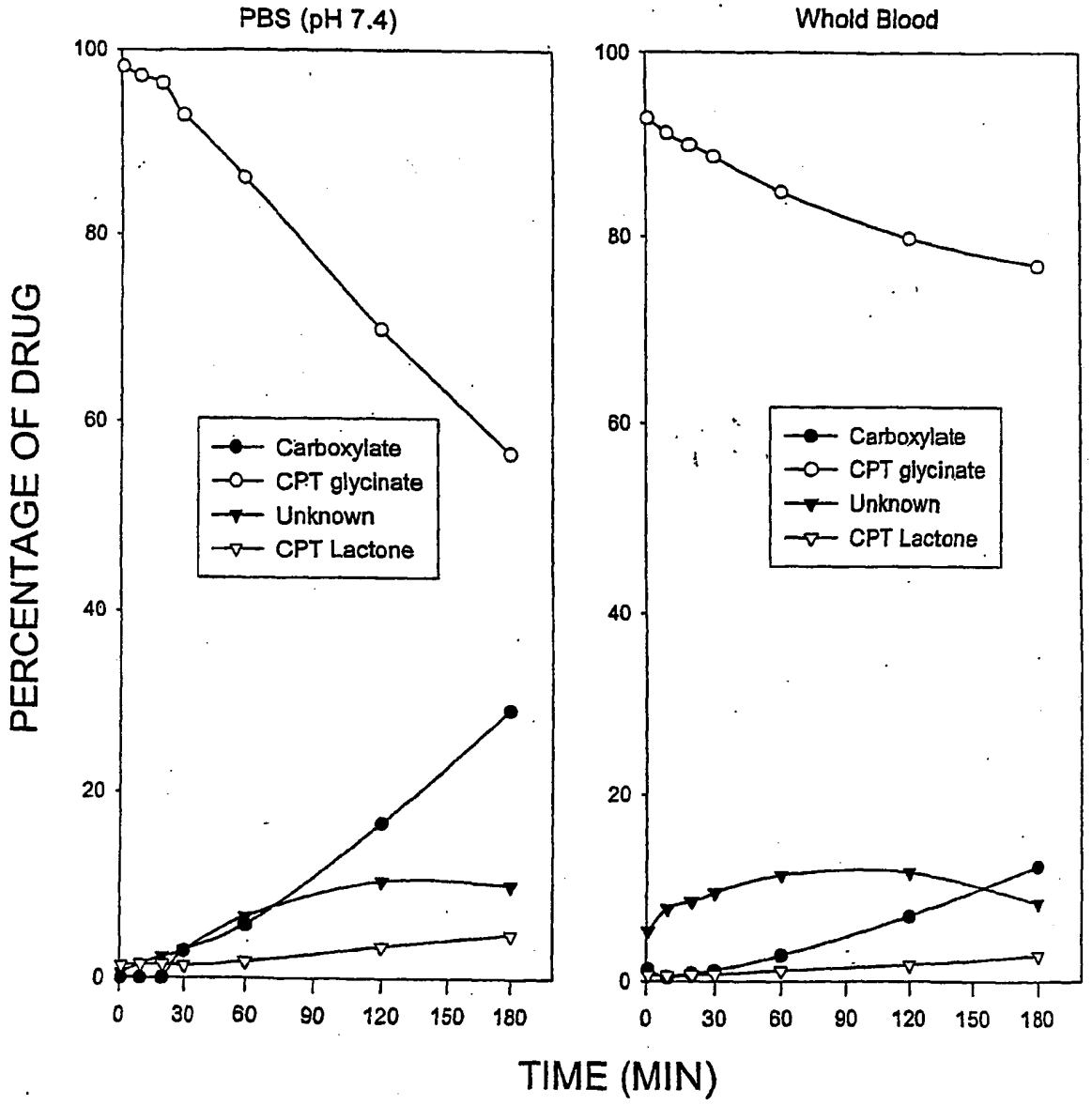

PBS (pH 7.4)
Whold Blood
PERCENTAGE OF DRUG
TIME (MIN)
Carboxylate
CPT glycinate
Unknown
CPT Lactone
0
20
40
60
80
100
0
30
60
90
120
150
180

Fig. 15

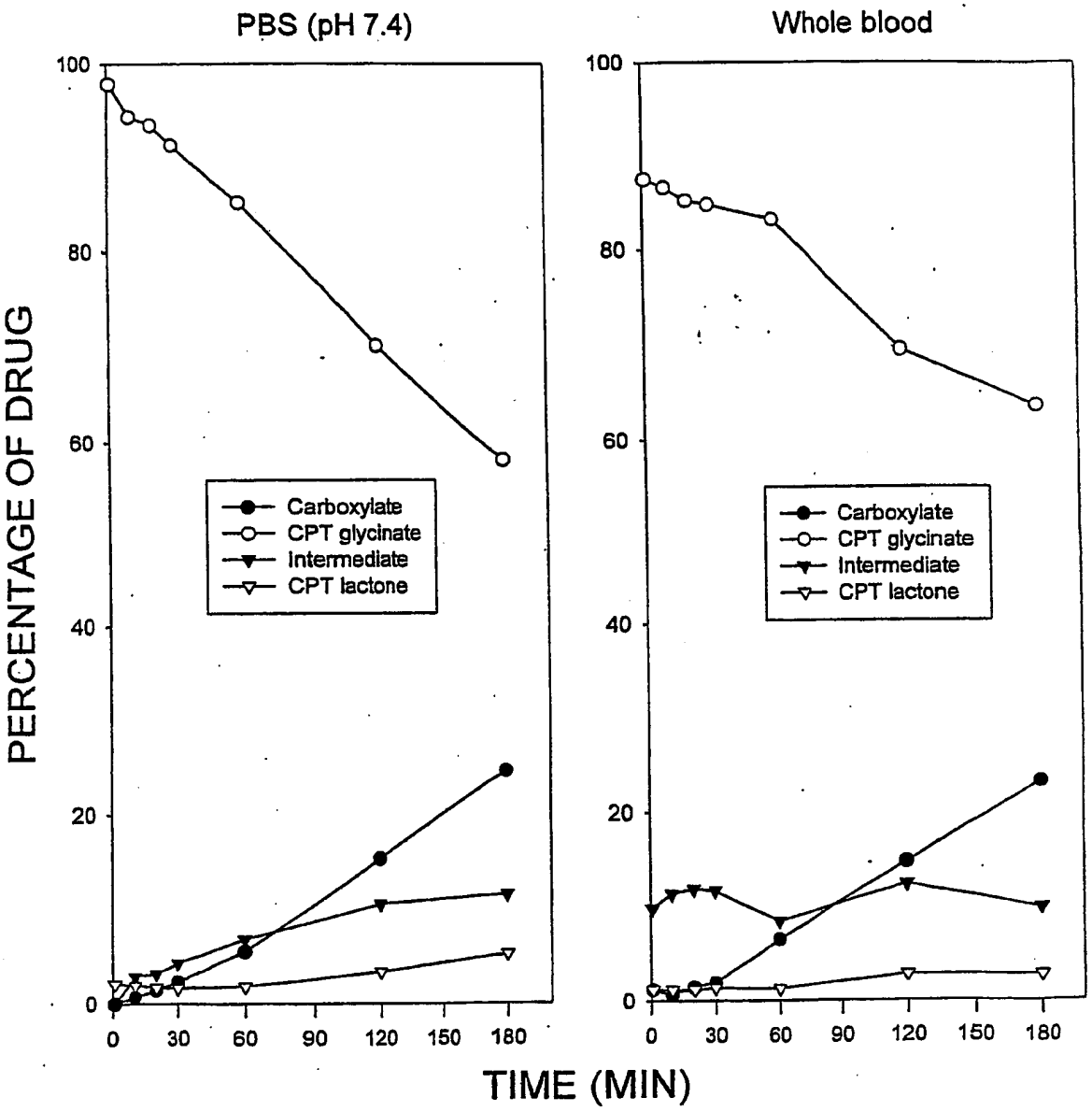

PBS (pH 7.4)
Whole blood
PERCENTAGE OF DRUG
TIME (MIN)
Carboxylate
CPT glycinate
Intermediate
CPT lactone
0
20
40
60
80
100
0
30
60
90
120
150
180

Fig. 16

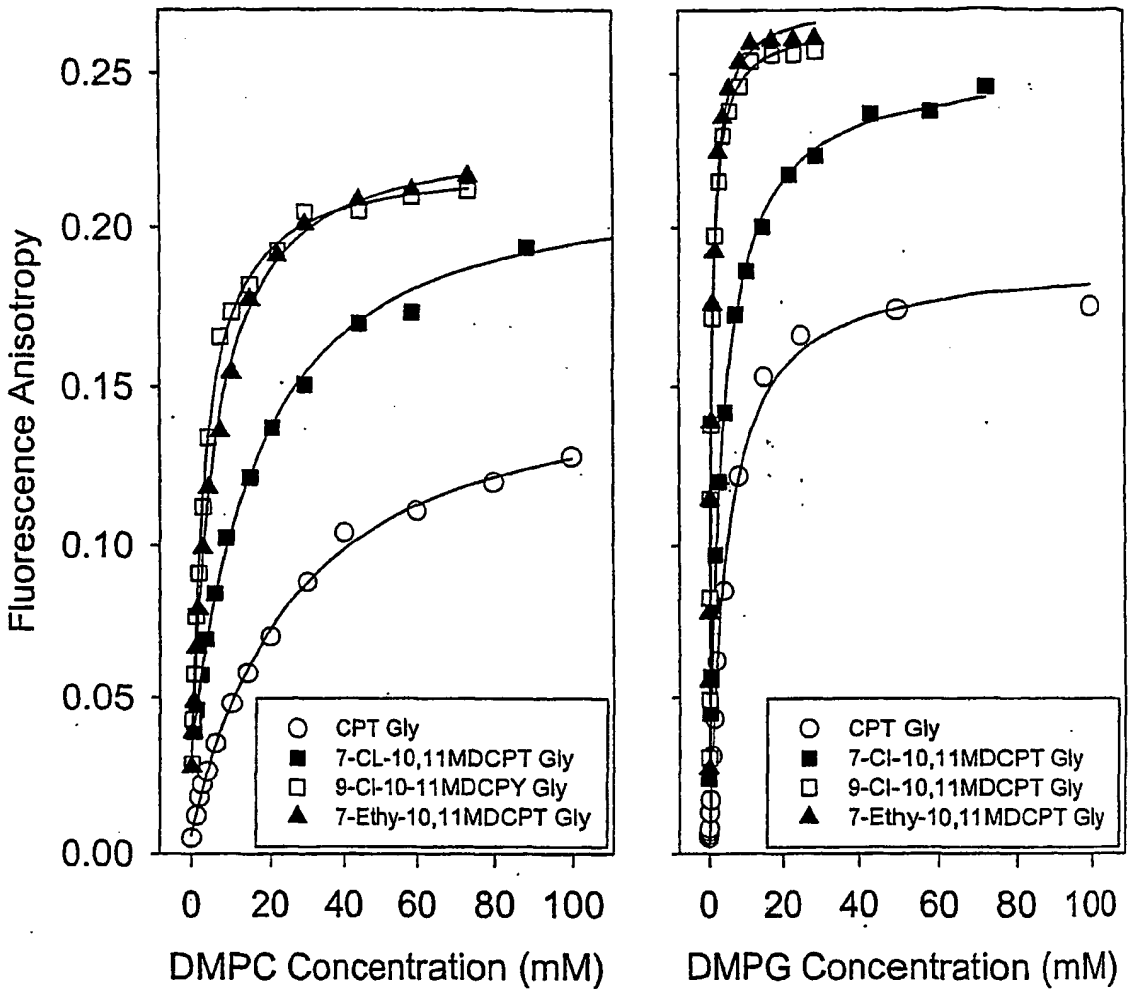

Fluorescence Anisotropy
0.25
0.20
0.15
0.10
0.05
0.00
0
20
40
60
80
100
DMPC Concentration (mM)
CPT Gly
7-CL-10,11MDCPT Gly
9-Cl-10-11MDCPY Gly
7-Ethy-10,11MDCPT Gly
DMPG Concentration (mM)
CPT Gly
7-Cl-10,11MDCPT Gly
9-Cl-10,11MDCPT Gly
7-Ethy-10,11MDCPT Gly

Fig. 17

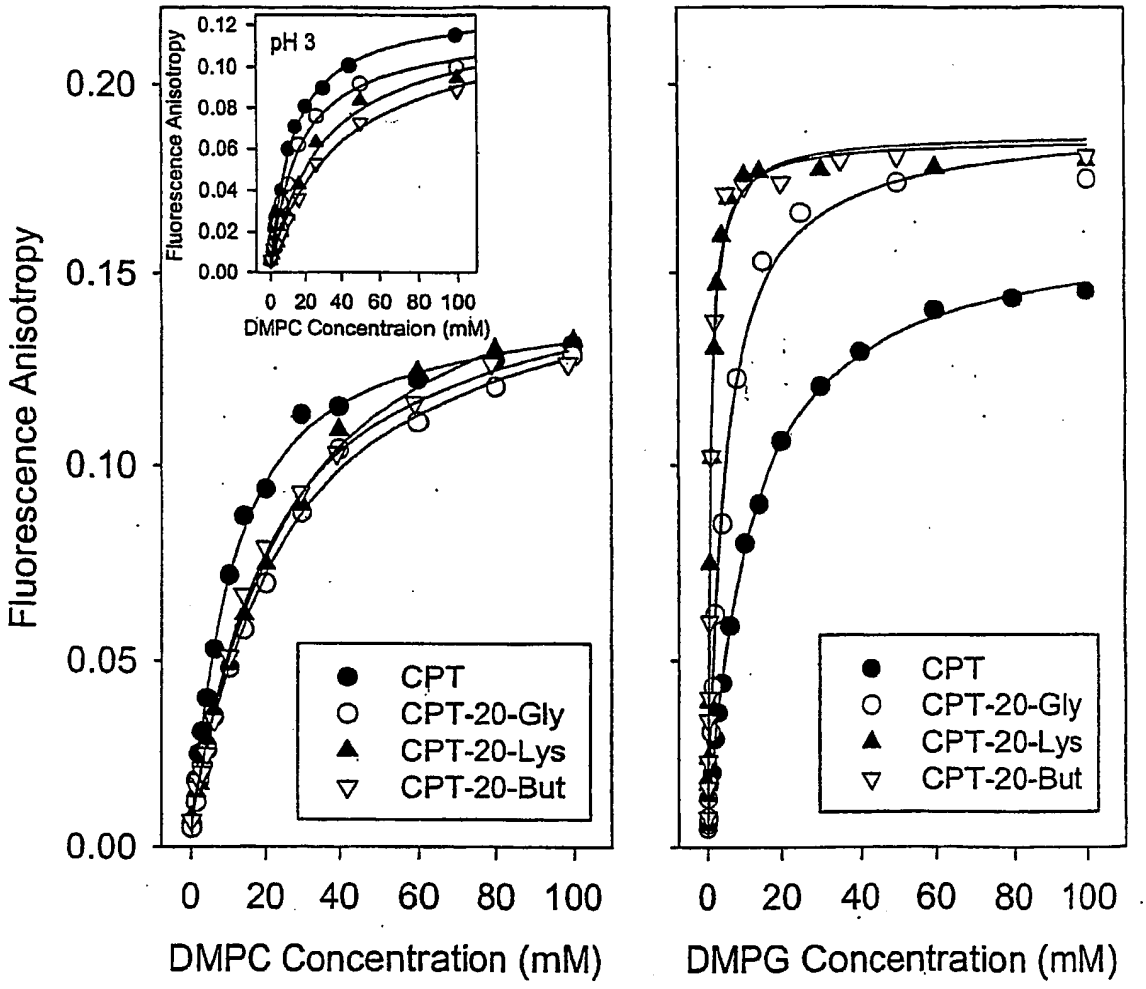

Fluorescence Anisotropy
0.20
0.15
0.10
0.05
0.00
0 20 40 60 80 100
DMPC Concentration (mM)
pH 3
Fluorescence Anisotropy
0.12
0.10
0.08
0.06
0.04
0.02
0.00
0 20 40 60 80 100
DMPC Concentraion (mM)
CPT
CPT-20-Gly
CPT-20-Lys
CPT-20-But
0 20 40 60 80 100
DMPG Concentration (mM)
CPT
CPT-20-Gly
CPT-20-Lys
CPT-20-But

Fig. 18

lipophilic DB67 glycinate ester.

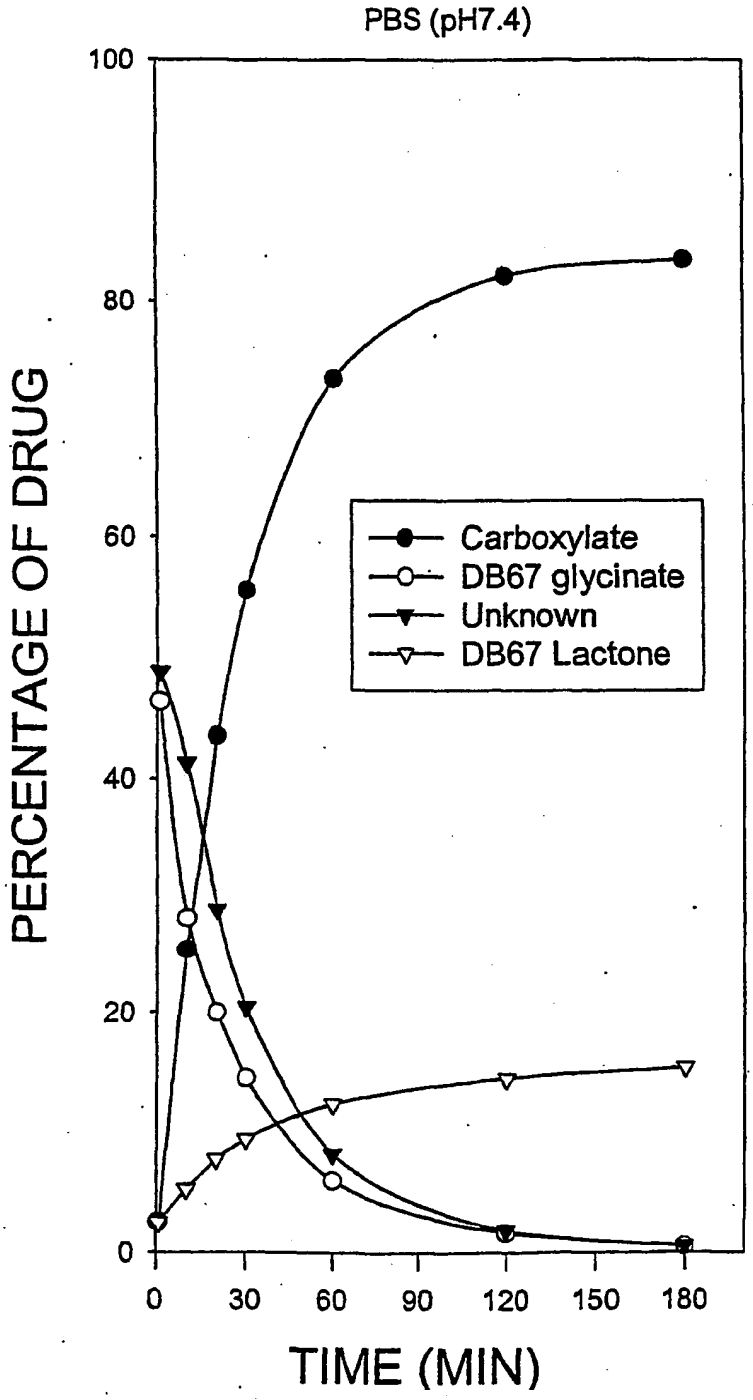


Fig. 19

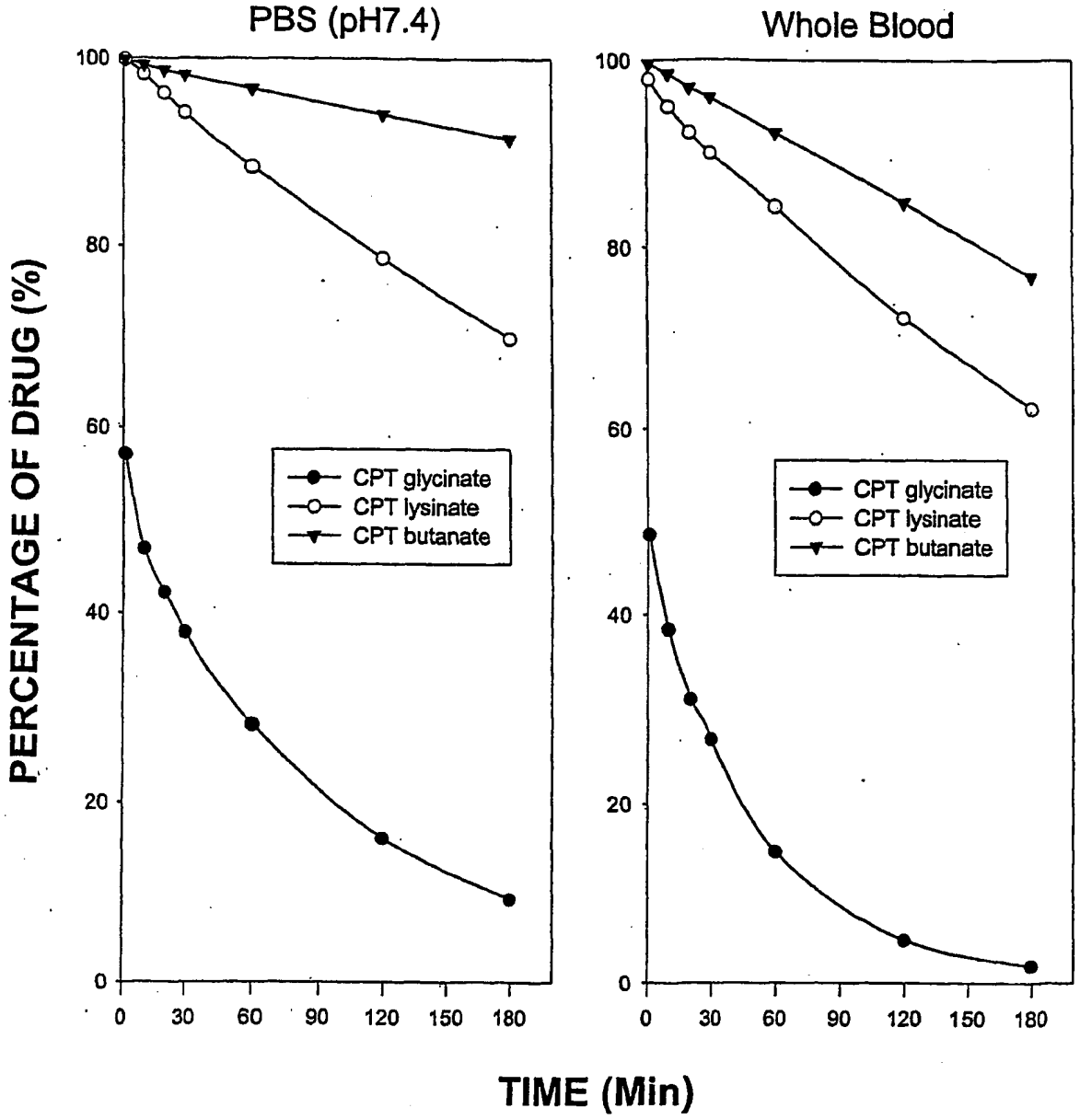

PBS (pH7.4)
Whole Blood
PERCENTAGE OF DRUG (%)
TIME (Min)
CPT glycinate
CPT lysinate
CPT butanate
0
20
40
60
80
100
0
30
60
90
120
150
180

Fig. 20

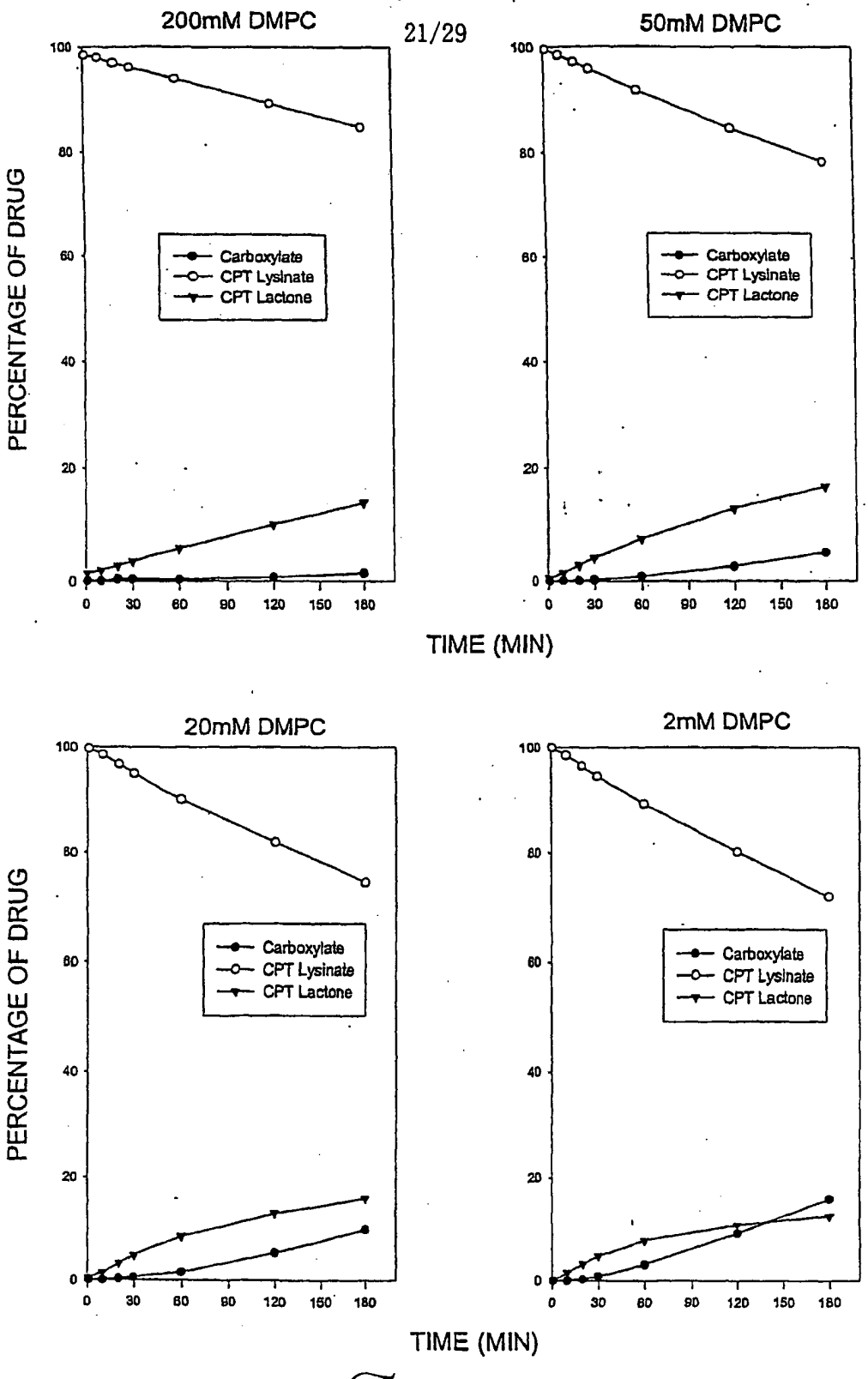

200mM DMPC
50mM DMPC
20mM DMPC
2mM DMPC
PERCENTAGE OF DRUG
TIME (MIN)
Carboxylate
CPT Lysinate
CPT Lactone
0
20
40
60
80
100
0
30
60
90
120
150
180

Fig. 21

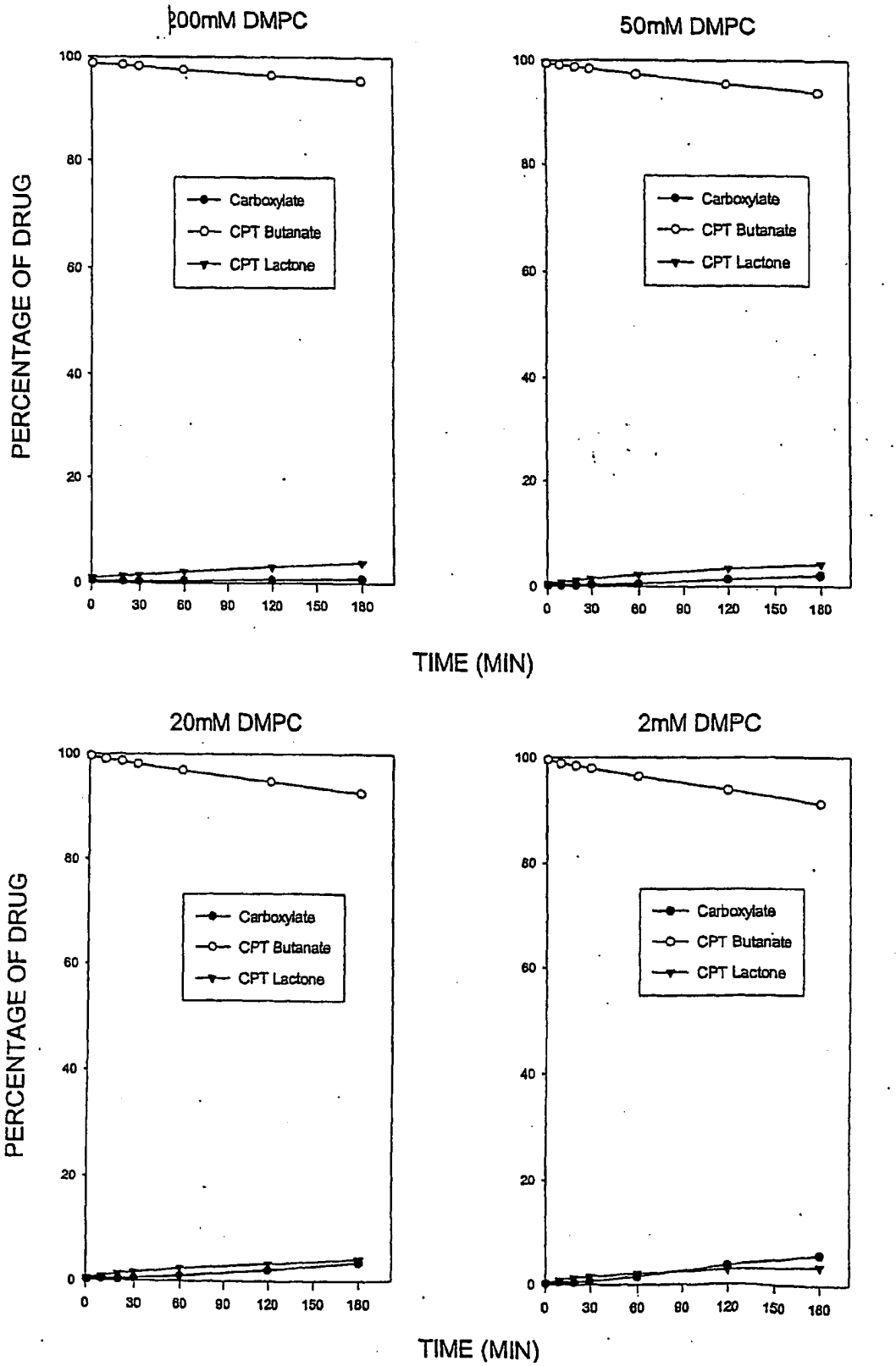

200mM DMPC
50mM DMPC
20mM DMPC
2mM DMPC
PERCENTAGE OF DRUG
TIME (MIN)
Carboxylate
CPT Butanate
CPT Lactone
0
20
40
60
80
100
0
30
60
90
120
150
180

Fig. 22

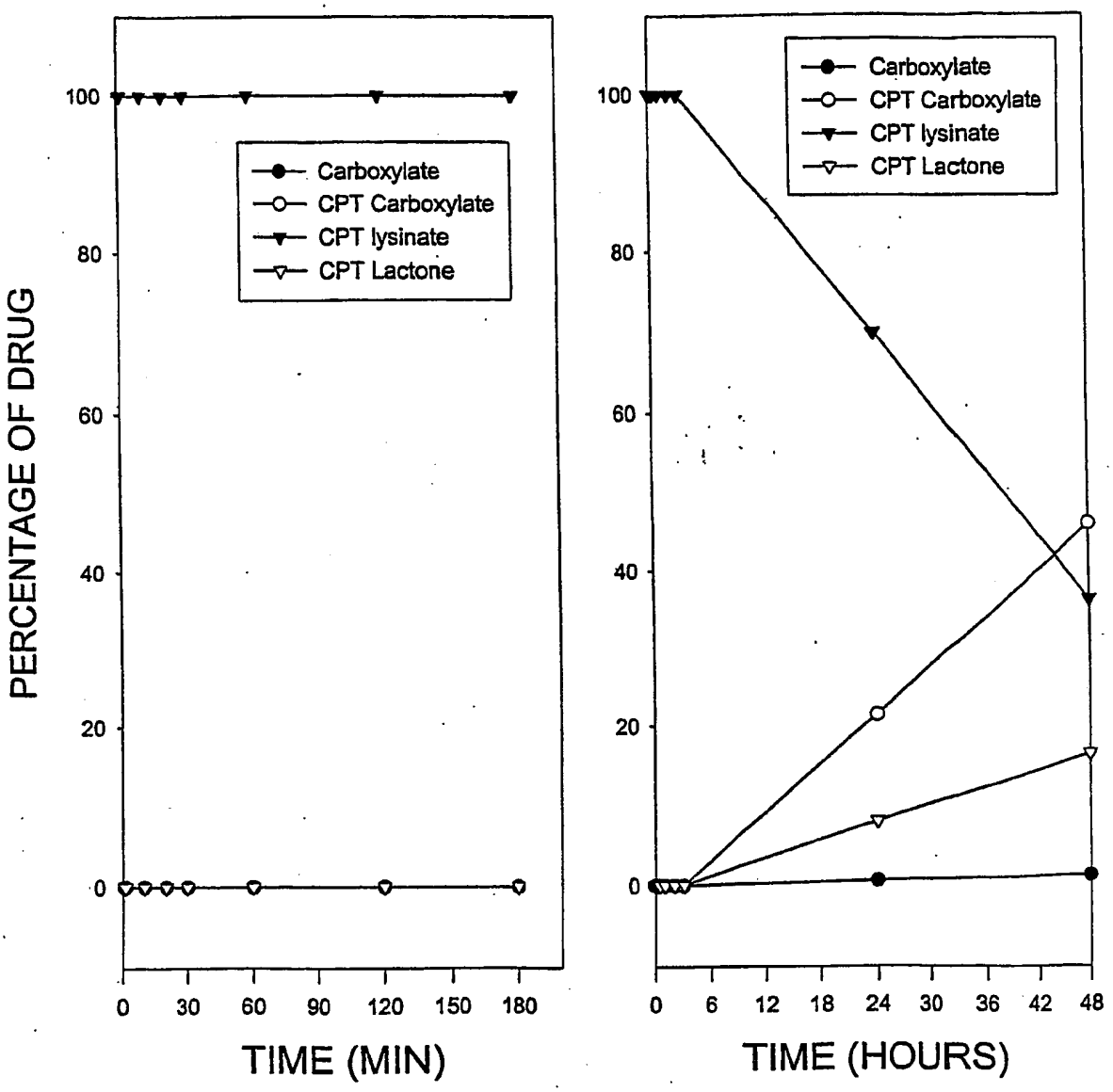

PERCENTAGE OF DRUG
100
80
60
40
20
0
Carboxylate
CPT Carboxylate
CPT lysinate
CPT Lactone
0 30 60 90 120 150 180
TIME (MIN)
0 6 12 18 24 30 36 42 48
TIME (HOURS)

Fig. 23

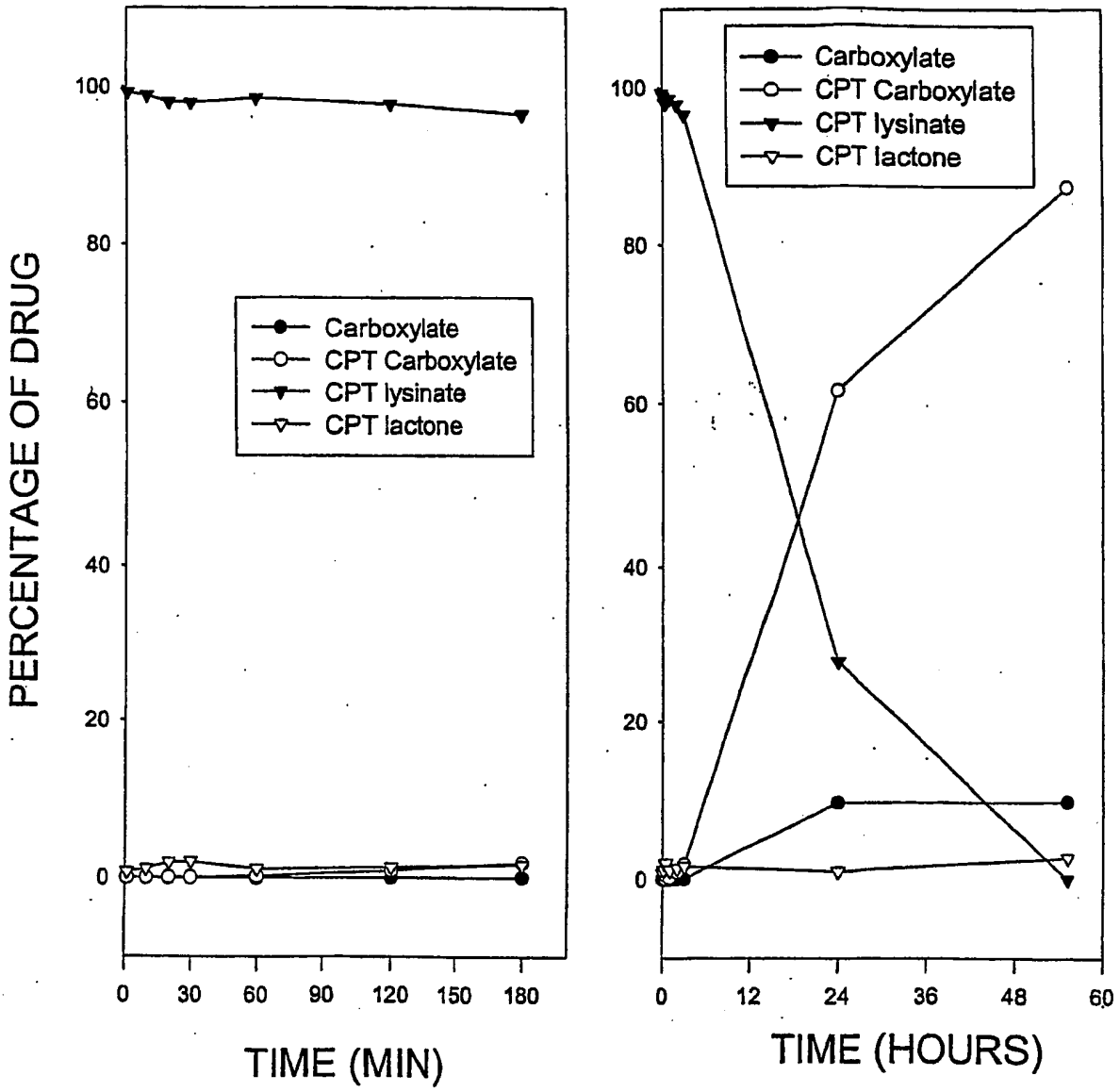

PERCENTAGE OF DRUG
100
80
60
40
20
0
Carboxylate
CPT Carboxylate
CPT lysinate
CPT lactone
0 30 60 90 120 150 180
TIME (MIN)
0 12 24 36 48 60
TIME (HOURS)

Fig. 24

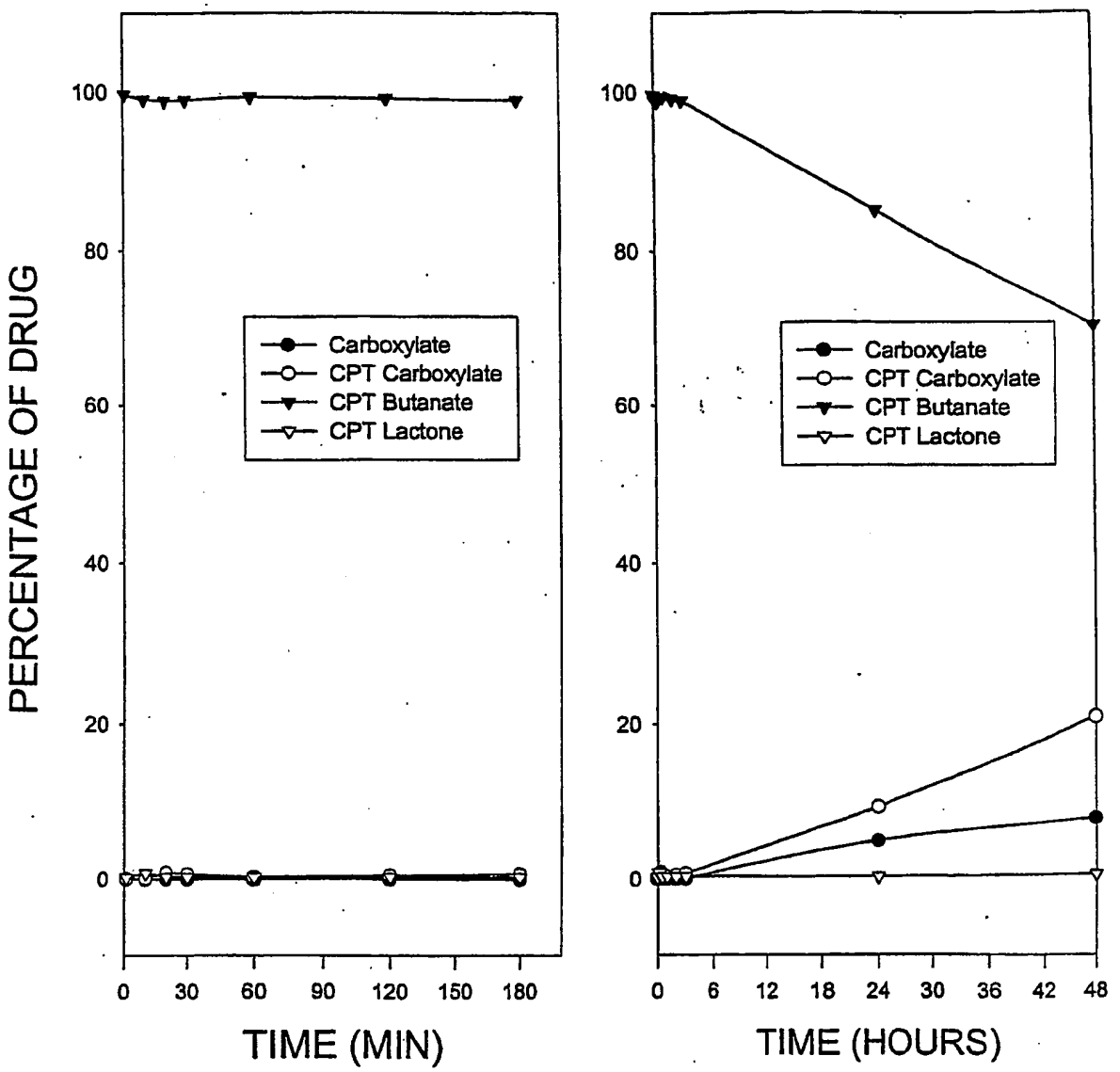

PERCENTAGE OF DRUG
100
80
60
40
20
0
Carboxylate
CPT Carboxylate
CPT Butanate
CPT Lactone
0 30 60 90 120 150 180
TIME (MIN)
0 6 12 18 24 30 36 42 48
TIME (HOURS)

Fig. 25

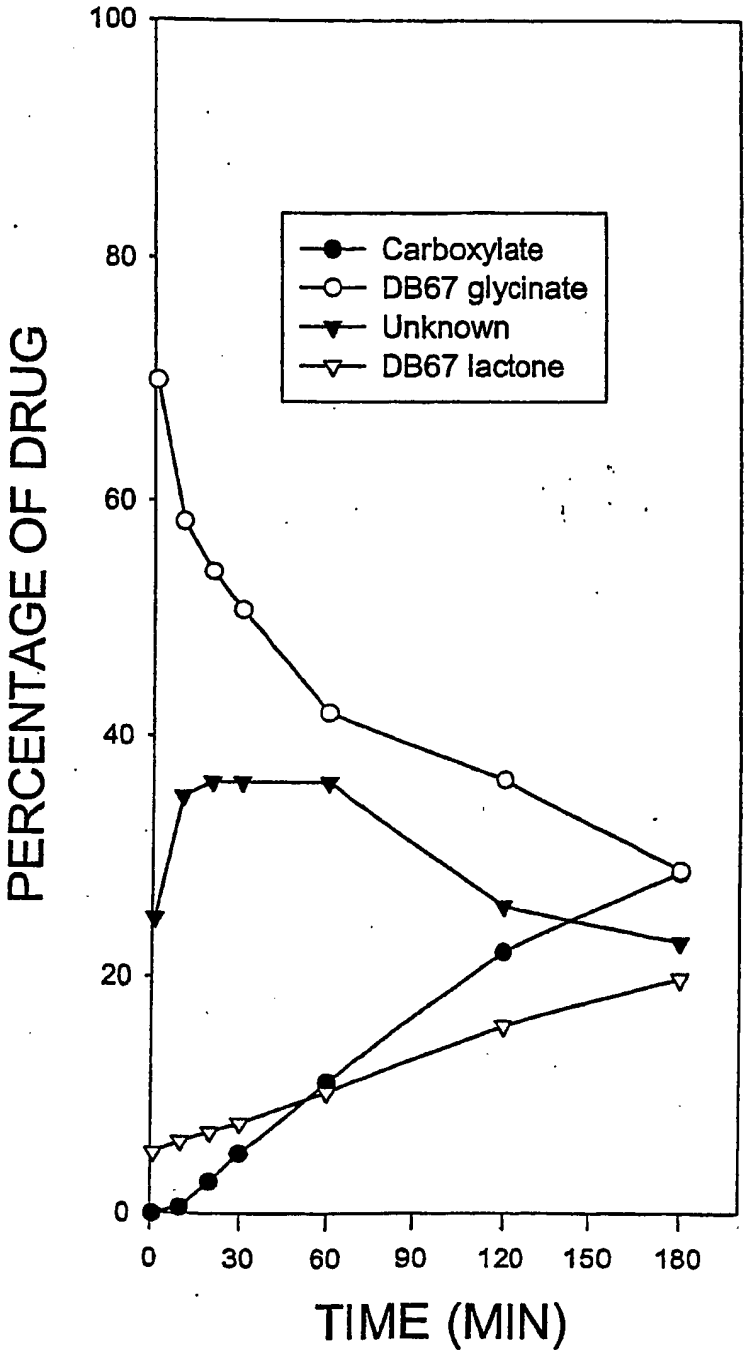

100
80
60
40
20
0
PERCENTAGE OF DRUG
Carboxylate
DB67 glycinate
Unknown
DB67 lactone
0
30
60
90
120
150
180
TIME (MIN)

Fig. 26

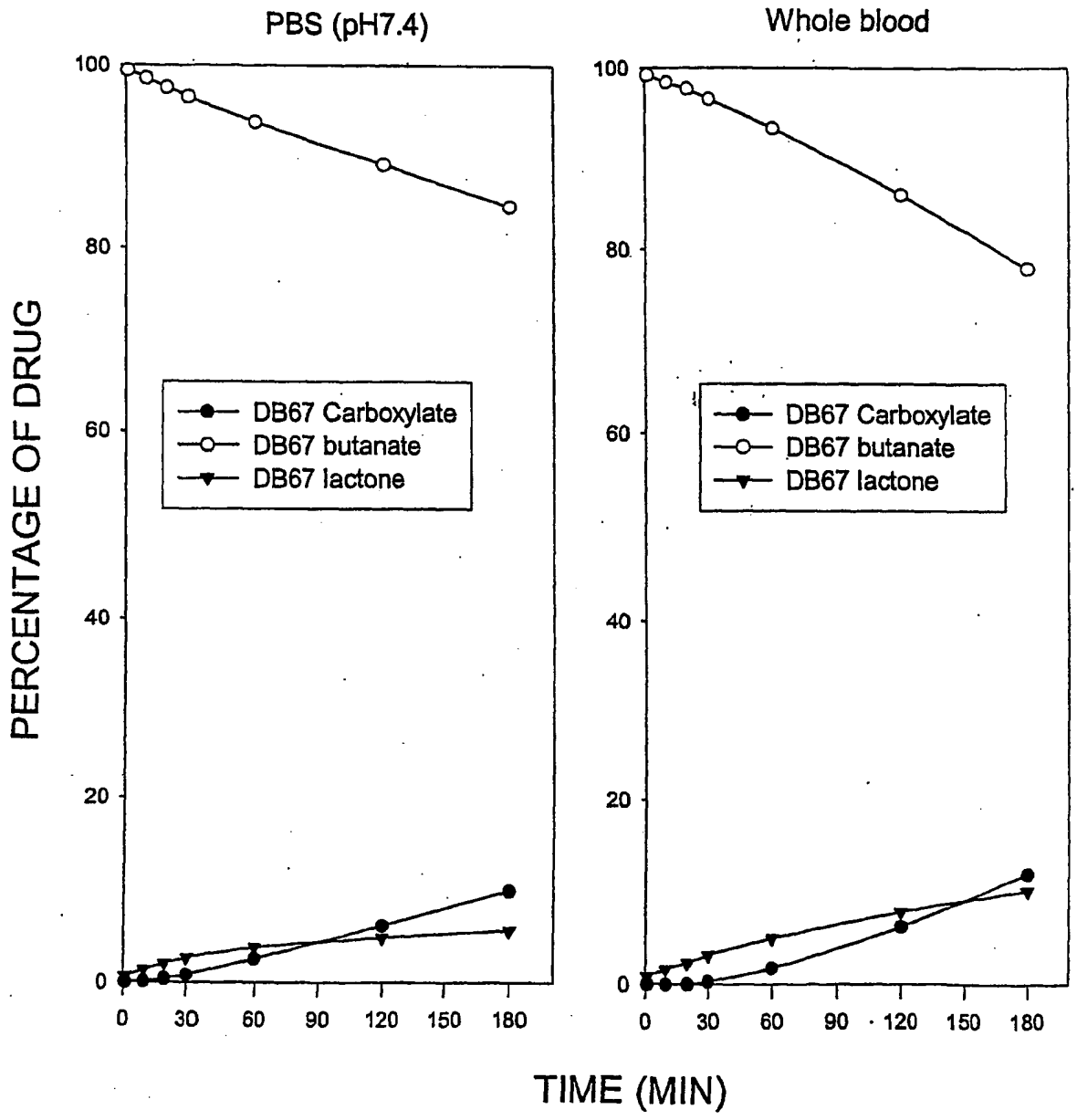

PBS (pH7.4)
Whole blood
PERCENTAGE OF DRUG
TIME (MIN)
DB67 Carboxylate
DB67 butanate
DB67 lactone
0
20
40
60
80
100
0
30
60
90
120
150
180

Fig. 27

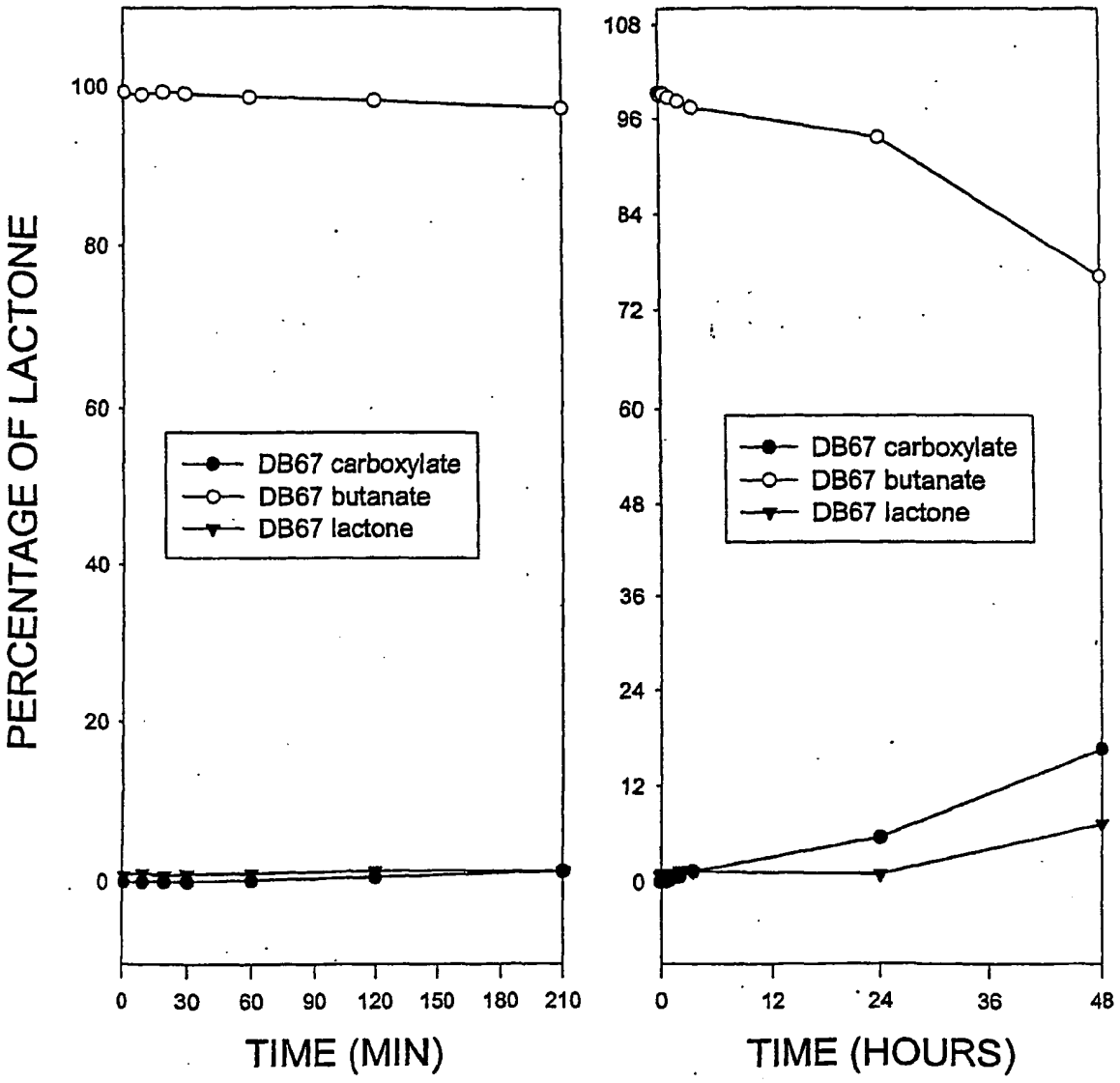
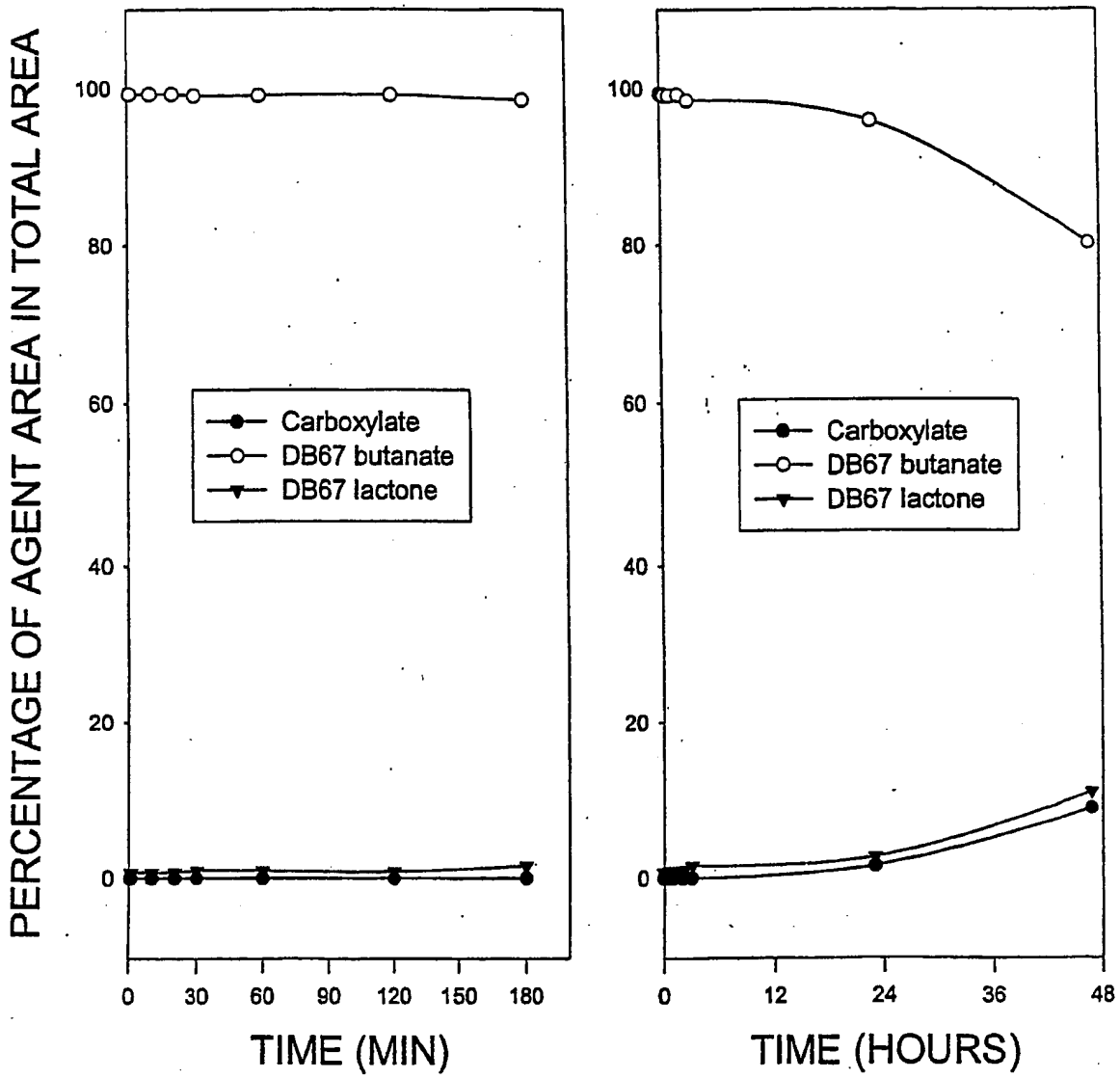

PERCENTAGE OF LACTONE
100
80
60
40
20
0
DB67 carboxylate
DB67 butanate
DB67 lactone
0
30
60
90
120
150
180
210
TIME (MIN)
108
96
84
72
60
48
36
24
12
0
DB67 carboxylate
DB67 butanate
DB67 lactone
0
12
24
36
48
TIME (HOURS)

Fig. 28



Stability of DB67 butanate liposome (112900-1)
in whole blood at 37°C

Fig. 29